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THE DIAGNOSIS OF RETINOPATHY BY FLUORESCEIN ANGIOGRAPHY IN LATENT DIABETES

BY

BIRGITTA ZETTERSTRÖM and MAGNUS GJÖTTERBERG

Four cases of diabetes are reported in which there was no ophthalmoscopic evidence of retinopathy and diabetes remained obscure until fluorescein angiography disclosed signs of retinopathy and multiple microaneurysms. The diagnosis of latent diabetes was made in three of these patients whose fasting blood sugar was normal but the glucose tolerance test was abnormal. In the fourth patient, whose fasting blood sugar and glucose tolerance test were abnormal, the diagnosis of manifest diabetes was made.

Key words: microaneurysm – latent diabetes – fluoresceinangiography – oscillatory potentials

Retinal microaneurysms are sac-like dilatations of the walls of the retinal capillaries. Their diameter ranges from 20μ to 90μ and they usually involve the capillaries in the inner nuclear layer. They generally co-exist with diabetic retinopathy but are encountered also in conditions such as retinal vein occlusion, pulseless disease, carotis stenosis, macroglobulinaemia, pernicious anaemia, glaucoma, hypertension, arteriosclerosis, Eales disease and chorioretinitis. In exceptional cases they involve the capillaries in the peripheral parts of the retina in the absence of any other signs or symptoms of systemic disease. According to Wise (1956) these small saccular dilatations of the retinal capillary walls are not true aneurysms but are arrested attempts at retinal neovascula-

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Four cases of diabetes are reported in which there was no ophthalmoscopic evidence of retinopathy and diabetes remained obscure until fluorescein angiography disclosed signs of ret nopathy and multiple microaneurysms The diagnosis of latent diabetes was made in three of these patients whose fasting blood sugar was normal but the glucose tolerance test was abnormal in the fourth patient whose fasting blood sugar and glucose tolerance test were abnormal the diagnosis of manifest diabetes was made

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Retinal microaneurysms are sac like dilatations of the walls of the retinal capillaries Their diameter ranges from 20 μ to 90μ and they usually involve the capillaries in the inner nuclear layer They generally co exist with diabetic retinopathy but are encountered also in conditions such as retinal vein occlusion pulseless disease carotis stenosis macroglobulinaemia pernicious anaemia glaucoma hypertension arteriosclerosis Fales disease and chorioretinitis In exceptional cases they involve the capillaries in the peripheral parts of the retina in the absence of any other signs or symptoms of systemic disease According to Wise (1956) these small saccular dilatations of the retinal capillary walls are not true aneurysms but are arrested attempts at retinal neovascula

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yellow spots resembling hard exudates in type were seen in the macula region of both eyes The intra ocular pressures were 14 mmHg The visual fields showed no ab normality

Fluoresceinangiography A large number of closely approximated microaneurysms were clearly outlined in the left eye in th arterio venous phase The small yellow spots resembling hard exudates which were seen through the ophthalmoscope did not fill with the dye (Fig 2) Fig 1 shows the left fundus before the dye reached the blood vessels

The value of the *oscillatory potentials* of the electroretinograms obtained from the right and left eye was 280 μV Blood pressure was 140/80 mmHg Radiography of the lungs accessory sinuses sacro iliac joints and stomach showed no abnormality

Laboratory findings Hb was 14.3 g/100 ml Haematocrit was 42 per cent The differen tial blood count was normal The sedimentation rate was 4 mm/hour The erythrocytes showed no abnormality The toxoplasmosis dye test (Sabin Feldman) was negative < 1/10 The blood lipids level was normal There was no glycosuria or proteinuria The fasting blood sugar was 71 mg % The glucose tolerance test was abnormal the k-value being 0 703

Endrocrinologists report Apart from the abnormal glucose tolerance test there were no signs of diabetes *Diagnosis* Latent diabetes



Fig 1



Fig 2

Fig 1

Case 1 *Left fundus* Hard exudates are seen within the macula The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 2

Case 1 *Left fundus* In the arterio venous phase numerous microaneurysms are outlined

rization in response to anoxia of the retinal tissues. Cogan & Kuwabara (1967) argued against this view and considered intraretinal neovascularization to be extremely rare. They believed that the primary cause was the loss of pericytes by the capillaries thereby reducing the tone of the blood vessels and resulting in the formation of microaneurysms. Ashton (1967) expressed the view that the primary cause was the occlusion of capillaries associated with endothelial proliferation, which would result in arterio-venous shunts and formation of microaneurysms. Wessing (1968) investigated by fluorescein angiography the fundi of diabetics who had no ophthalmoscopic evidence of retinopathy and demonstrated that this procedure disclosed the presence of microaneurysms which are not visible ophthalmoscopically. A characteristic feature of the fluorescein angiogram of retinal microaneurysms is their arrangement in the central part of the fundus, where they crowd together primarily within or around the macula, and are seen either in the form of closely approximated red dots adjacent to capillaries with a feeding vessel or in the form of small round swellings at the blind end of capillaries resembling a pin head in shape. They fill with fluorescein in the arterial phase and empty simultaneously with the entire retinal vascular network, provided there is no leakage of dye. Drusen of Bruchs membrane may easily be confused with microaneurysms in the fluorescein angiogram because both are seen in the form of small fluorescent dots from the beginning of the arterial phase. According to Schikano & Shimizu (1968) the dye remains in the drusen much longer than in the retinal blood vessels. A careful check of their appearances in colour photographs further facilitates the differential diagnosis.

The paper reports four patients who attended this Department because of diffuse ocular symptoms. In order to determine the cause, their examination was extended to include fluorescein angiography to disclose the presence of retinal microaneurysms. Apart from the ocular symptoms the patients stated that they had no other noteworthy symptoms of systemic disease.

Case Reports

Case 1

The patient, a man aged 45 years, complained of reduced vision of one year's duration. Otherwise he enjoyed good health. There was no family history of diabetes.

Ophthalmological findings. The visual acuity of the right eye was 1.0 (−0.50 cyl 75°), that of the left was 0.9 (−0.[illegible] cyl 40°). The iris showed no abnormality. The lenses of both eyes were slightly opaque. The retinal blood vessels showed no abnormality. There was no ophthalmoscopic evidence of haemorrhage or microangiopathy. Small

yellow spots resembling hard exudates in type were seen in the macula region of both eyes The intra ocular pressures were 14 mmHg The visual fields showed no ab normality

Fluoresceinangiography A large number of closely approximated microaneurysms were clearly outlined in the left eye in the arterio venous phase The small yellow spots resembling hard exudates *which were seen through the ophthalmoscope did not* fill with the dye (Fig 2) Fig 1 shows the left fundus before the dye reached the blood vessels

The value of the *oscillatory potentials* of the electroretinograms obtained from the right and left eye was 280 μV Blood pressure was 140/80 mmHg Radiography of the lungs accessory sinuses *sacro iliac joints and stomach showed no abnormality*

Laboratory findings Hb *was 14 3 g/100 ml Haematocrit was 42 per cent The differen* tial blood count was normal The sedimentation rate was 4 mm/hour The erythrocytes showed no abnormality The toxoplasmosis dye test (Sabin Feldman) was negative < 1/10 The blood lipids level was normal There was no glycosuria or proteinuria. The fasting blood sugar was 71 mg % The glucose tolerance test was abnormal the *k-value being 0 63*

Endrocrinologists report Apart from the abnormal glucose tolerance test there were no signs of diabetes *Diagnosis* Latent diabetes



Fig 1



Fig 2

Fig 1

Case 1 Left fundus Hard exudates are seen within the macula The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 2

Case 1 Left fundus In the arterio venous phase numerous *microaneurysms are outlined*

Case 2

The patient a man 43 years of age attended because of impaired vision associated with physical exercise which he had experienced for about one year Otherwise he had enjoyed good health His mother had diabetes

Ophthalmological findings Visual acuity was 1 0 in both eyes The media were clear The iris showed no abnormality The retinal blood vessels appeared to be normal There was no evidence of haemorrhages or microangiopathy The intra ocular pressures were 19 mmHg The visual fields showed no defects

Fluorescein angiography Fig 3 shows the right fundus before the dye reached the blood vessels In the arterio venous phase a relatively large number of microaneurysms were outlined (Fig 4) In order to investigate whether there was objective evidence of impaired vision in association with exercise he was submitted to the exercise test on the bicycle ergometer Immediately after completion of the test both eyes were examined by fluorescein angiography The findings were identical with those on the previous occasion There was no evidence of leakage of dye which would have explained the subjective symptoms of reduced visual acuity in association with physical exercise

The clinical standardized electroretinogram according to Karpe (1945) elicited on the right and left eye showed readings of 0.8? mV and 0.40 mV respectively The values of the *oscillatory potentials* were 340 μV on both sides

Blood pressure was 120/80 mmHg The electrocardiogram was normal

Laboratory findings Hb was 15.7 g/100ml Haematocrit was 40 per cent The differential blood count was normal *The sedimentation rate was 3 mm/hour* The erythro



Fig 3



Fig 4

Fig 3

Case 2 Right fundus The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 4

Case 2 Right fundus Numerous microaneurysms are outlined in the arterio venous phase

cytes showed no abnormality The blood lipids were normal There was no evidence of glycosuria or proteinuria The fasting blood sugar was 10 mg % The glucose tolerance test was abnormal the k value being 0 752 *Endocrinologists report* Apart from the abnormal values shown by the glucose tolerance test there were no signs of diabetes *Dia nosis* Latent diabetes

Case 3

The patient a man aged 51 years attended on account of impairment of the visual acuity of his left eye which he had noticed in the past year The degree of visual im pairment was variable He had a history of dislocation of the 5th lumbar vertebra and pain in the back associated with physical exercise About one year previously he had undergone a medical check up The urine contained neither albumin nor sugar and blood lipids and Hb were n rmal on that occasion There was no family history of diabetes

Ophthalmological findings The visual acuity of the right eye was 1 0 that of the left 0 7 (−0 5 cyl 180°) The media were clear The iris showed no abnormality The right fundus was normal In the left fundus about one disc diameter superior and nasally to the disc there was a small scar from retinochoroiditis The blood vessels sho wed no abnormality There was no evidence of haemorrhages or microangiopathy The intra ocular pressures were 19 mmHg The visual fields showed no abnormality



Fig 5



Fig 6

Fig 5

Case 3 Left fundus The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 6

Case 3 Fundus of the left eye Numerous microaneurysms are outlined early in the arterio venous phase

Case 2.

The patient, a man 43 years of age, attended because of impaired vision associated with physical exercise which he had experienced for about one year. Otherwise he had enjoyed good health. His mother had diabetes.

Ophthalmological findings. Visual acuity was 1.0 in both eyes. The media were clear. The iris showed no abnormality. The retinal blood vessels appeared to be normal. There was no evidence of haemorrhages or microangiopathy. The intra ocular pressures were 19 mmHg. The visual fields showed no defects.

Fluorescein angiography. Fig. 3 shows the right fundus before the dye reached the blood vessels. In the arterio venous phase a relatively large number of microaneurysms were outlined (Fig. 4). In order to investigate whether there was objective evidence of impaired vision in association with exercise, he was submitted to the exercise test on the bicycle ergometer. Immediately after completion of the test both eyes were examined by fluorescein angiography. The findings were identical with those on the previous occasion. *There was no evidence of leakage of dye which would have explained* the subjective symptoms of reduced visual acuity in association with physical exercise.

The clinical standardized electroretinogram according to Karpe (1945) elicited on the right and left eye showed readings of 0.87 mV and 0.40 mV respectively. The values of the *oscillatory potentials* were 340 μV on both sides.

Blood pressure was 120/80 mmHg. The electrocardiogram was normal.

Laboratory findings. Hb was 15.7 g/100ml. Haematocrit was 40 per cent. The differential blood count was normal. The sedimentation rate was 3 mm/hour. The erythro-

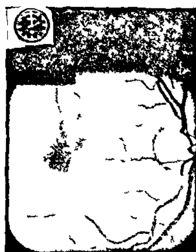


Fig. 3



Fig. 4

Fig. 3

Case 2. Right fundus. The dye has not yet reached the blood vessels. No evidence of microangiopathy.

Fig. 4

Case 2. Right fundus. Numerous microaneurysms are outlined in the arterio venous phase.

cytes showed no abnormality The blood lipids were normal There was no evidence of glycosuria or proteinuria The fasting blood sugar was 0 ng% The glucose tolerance test was abnormal the k value being 0 75? *Endocrinologists report* Apart from the abnormal values shown by the glucose tolerance test there were no signs of diabetes *Diagnosis* Latent diabetes

Case 3

The patient a man aged 51 years attended on account of impairment of the visual acuity of his left eye which he had noticed in the past year The degree of visual impairment was variable He had a history of dislocation of the 5th lumbar vertebra and pain in the back associated with physical exercise About one year previously he had undergone a medical check up The urine contained neither albumin nor sugar and blood lipids and Hb were normal on that occasion There was no family history of diabetes

Ophthalmological findings The visual acuity of the right eye was 1 0 that of the left 0 7 (-0 5 cyl 180) The media were clear The iris showed no abnormality The right fundus was normal In the left fundus about one disc diameter superior and nasally to the disc there was a small scar from retinochoroiditis The blood vessels showed no abnormality There was no evidence of haemorrhages or microangiopathy The intra ocular pressures were 1? mmHg The visual fields showed no abnormality



Fig 5



Fig 6

Fig 5

Case 3 Left fundus The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 6

Case 3 Fundus of the left eye Numerous microaneurysms are outlined early in the arterio venous phase

Case 2.

The patient a man 48 years of age attended because of impaired vision associated with physical exercise which he had experienced for about one year Otherwise he had enjoyed good health His mother had diabetes

Ophthalmological findings Visual acuity was 1.0 in both eyes The media were clear The iris showed no abnormality The retinal blood vessels appeared to be normal There was no evidence of haemorrhages or microangiopathy The intra ocular pressures were 19 mmHg The visual fields showed no defects

Fluorescein angiography Fig 3 shows the right fundus before the dye reached the blood vessels In the arterio venous phase a relatively large number of microaneurysms were outlined (Fig 4) In order to investigate whether there was objective evidence of impaired vision in association with exercise he was submitted to the exercise test on the bicycle ergometer Immediately after completion of the test both eyes were examined by fluorescein angiography The findings were identical with those on the previous occasion There was no evidence of leakage of dye which would have explained the subjective symptoms of reduced visual acuity in association with physical exercise

The clinical standardized electroretinogram according to Karpe (1945) elicited on the right and left eye showed readings of 0.3[illegible] mV and 0.40 mV respectively The values of the *oscillatory potentials* were 340 µV on both sides

Blood pressure was 120/80 mmHg The electrocardiogram was normal

Laboratory findings Hb was 15.[illegible] g/100ml Haematocrit was 40 per cent The differential blood count was normal The sedimentation rate was 3 mm hour The erythro



Fig 3



Fig 4

Fig 3

Case 2 Right fundus The dye has not yet reached the blood vessels No evidence of microangiopathy

Fig 4

Case 2 Right fundus Numerous microaneurysms are outlined in the arterio venous phase

Case 4

The patient a woman 54 years of age attended because of impairment of vision for the last two years She had had mild symptoms of arthritis for several years and her urine was occasionally found to contain sugar Otherwise she enjoyed good health Her mother had diabetes

Ophthalmological findings The visual acuity of the right eye was 0 5 (+1 2ɔ cyl 180°) that of the left being 1 0 (+0 5 cyl 180°) The media were clear The iris showed no abnormality The left fundus appeared to be normal In the right fundus a slight swelling of the retina within a light relatively sharply demarcated area around the macula was identified There was no evidence of haemorrhages or microangiopathy Th intra ocular pressures were 14 mm/Hg The visual fields showed no abnormality

Fluorescein angiography Numerous microaneurysms were outlined in the posterior pole of the right eye and there was evidence of diffuse leakage of dye around the l ght area seen ophthalmoscopically (Figs 9 10) A number of microaneurysms around the macula were also outlined in the fluorescein angiogram of the left eye (Figs 11 12)

The clinical electroretinograms elicited from the right and left eye showed readings of 0 ?5 mV and 0 ?7 mV respectively The value of the *oscillatory potentials recorded* in the electroretinogram obtained from the right eye was ?40 μV the corresponding value recorded in that elicited on the left eye was 300 μV

Blood pressure was 140 90 mmHg



Fig 9



Fig 10

Fig 9

Case 4 Right fundus A slight swelling with lighter zone in the centre of the *fundus* is seen The dye has not reached the blood vessels

Fig 10

Case 4 R ght fundus Arterio veno s phase Numerous microaneurysms are outlined Evidence of slight leakage of dye around the central area.

Fluorescein angiography Numerous microaneurysms were outlined in the left fundus at the beginning of the arterio venous phase (Fig 6) These were not visible in the black and white fundus photographs (Fig 5) As in the fluorescein angiogram of the left eye microaneurysms were also outlined in the right eye (Fig 7) and as on the previous occasion they were not visible in the black and white fundus photographs (Fig 8)

The values of the *oscillatory potentials* in the electroretinograms obtained from the right and left eye were 250 μV and 280 μV respectively

Blood pressure was 130 90 mmHg

Radiological findings The mucosa of the frontal sinuses was thickened The lungs showed no abnormality The lumbo sacral spine showed degeneration of the disc LV SI

Laboratory findings Hb was 16 1 g/100 ml Haematocrit was 49 per cent The differential blood count was normal The sedimentation rate was 4 mm/hour The appearance of the erythrocytes was normal The toxoplasmosis test (Sabin Feldman) was negative < 1/10 The blood lipids were normal There was no glycosuria or proteinuria The fasting blood sugar on one occasion was 44 mg % and on a later occasion 59 mg % The glucose tolerance test was abnormal the k value being 0 ,05 *Endocrinologists report* Apart from the abnormal values shown by the glucose tolerance test there were no signs of diabetes *Diagnosis* Latent diabetes



Fig 7



Fig 8

Fig 7

Case 3 Right fundus Numerous microaneurysms are outlined late in the arteriovenous phase

Fig 8

Case 3 Right fundus The dye has not yet reached the blood vessels No evidence of microangiopathy

The results of the investigations of the incidence of retinopathy in juvenile diabetics i e patients who develop diabetes before 15 or 16 years of age are comparable Retinopathy has been reported to co exist with diabetes from 5 to 9 years duration in 10 per cent of cases with diabetes of 15 years duration in 50 per cent of cases and with diabetes of 25 years duration or more it co existed in 80–90 per cent of cases (Kornerup 1955 White 1960 Larsson & Sterky 1962) In persons who developed diabetes before the age of 30 years and when the duration was shorter than 5 years the risk of retinopathy developing has been calculated to be 2 per cent per year rising to 13 per cent per year with diabetes of 14 years duration or more (Burditt et al 1968 Knowles et al 1965) Janert et al (1956) observed that in persons in whom diabetes appeared after the age of 40 years retinopathy developed as early as the first year of the presence of diabetes in 0.5 per cent of cases and Malins & Fitzgerald (1965) reported the corresponding percentage to be 0 6 per cent In persons who were between 40 and 60 years of age when diabetes was recognized retinopathy developed in the first year of the presence of diabetes in 5 per cent of cases (Lundbæk 1955 Kornerup 1958) It emerges that (i) retinopathy rarely develops in persons in whom diabetes appears before the age of 30 years and the duration is less than 5 years and (ii) if the diagnosis of diabetes is made in middle aged persons there is risk of retinopathy developing with diabetes of only short duration

Linner et al (1965) described a 60 year old woman with a family history of diabetes in whom a retinopathy of diabetic appearance and renal functional and ultrastructual changes compatible with those observed in diabetes developed Repeated investigations however failed to demonstrate obvious disturbances in glucose tolerance

Electroretinographic studies using a special technique which permits the recording of the oscillatory potentials of the electroretinogram have shown that they are often selectively reduced at a very early stage of diabetic retinopathy (Yonemura et al 1962 Sugita et al 1963 Simonsen 1965 Kojima et al 1966 Tassy 1966 Jacobsen et al 1967 Nakajima & Sugimachi 1966) Simonsen (1965) recorded the oscillatory potentials in a series of diabetics who ophthalmoscopically did not show any signs of retinopathy and observed reduced as well as increased oscillatory potentials He believed the electrophysiological disturbances to precede changes in the retina which were not yet visible ophthalmoscopically Fluorescein angiography might have permitted the early diagnosis of retinopathy in those cases

Fig 13 shows the values of the oscillatory potentials as recorded in the electroretinograms obtained from the eyes of the four patients discussed It is seen that the values of the oscillatory potentials are around the lower limit of

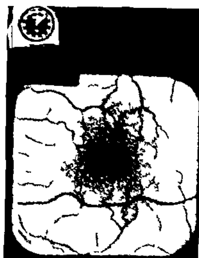


Fig 11

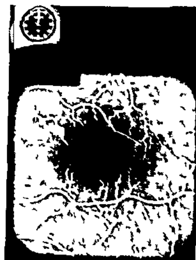


Fig 12

Fig 11
Case 4 Left fundus The dye has not reached the blood vessels No evidence of microangiopathy

Fig 12
Case 4 Left fundus Arterio venous phase Numerous microaneurysms are outlined

Laboratory findings Hb was 14 9 g/100 ml Haematocrit was 51 per cent The differential blood count was normal The sedimentation rate was 20 mm/hour The fasting blood sugar was 129 mg % The blood lipids were normal There was neither glycosuria nor proteinuria The glucose tolerance test was abnormal the k value being 0 559 *Endocrinologists report* Fasting hyperglycemia the glucose tolerance test showed abnormal values *Diagnosis* Manifest diabetes

Discussion

In order to throw some light on the causal relationship between diabetes and retinopathy factors such as age sex duration and treatment of diabetes have been studied These investigations have demonstrated that the most important factors involved in the origin of retinopathy are the age of the patients at the time of the appearance of the signs and symptoms of the diabetes and the duration of the disease Retinopathy is rare in diabetic children under 10 years of age irrespective of the duration of the diabetes (Forsyth & Payne 1956 Imerslund 1959 Cavner 1960 Unger 1960 Forsius 1964 Knowles et al 1965)

The results of the investigations of the incidence of retinopathy in juvenile diabetics i e patients who develop diabetes before 15 or 16 years of age are comparable Retinopathy has been reported to co exist with diabetes from 5 to 9 years duration in 10 per cent of cases with diabetes of 15 years duration in 50 per cent of cases and with diabetes of 25 years duration or more it co existed in 80–90 per cent of cases (Kornerup 1955 White 1960 Larsson & Sterky 1962) In persons who developed diabetes before the age of 30 years and when the duration was shorter than 5 years the risk of retinopathy developing has been calculated to be 2 per cent per year rising to 13 per cent per year with diabetes of 14 years duration or more (Burditt et al 1968 Knowles et al 1965) Janert et al (1956) observed that in persons in whom diabetes appeared after the age of 40 years retinopathy developed as early as the first year of the presence of diabetes in 0 5 per cent of cases and Malins & Fitzgerald (1965) reported the corresponding percentage to be 0 6 per cent In persons who were between 40 and 60 years of age when diabetes was recognized retinopathy developed in the first year of the presence of diabetes in 5 per cent of cases (Lundbæk 1955 Kornerup 1958) It emerges that (i) retinopathy rarely develops in persons in whom diabetes appears before the age of 30 years and the duration is less than 5 years and (ii) if the diagnosis of diabetes is made in middle aged persons there is risk of retinopathy developing with diabetes of only short duration

Linnér et al (1965) described a 60 year old woman with a family history of diabetes in whom a retinopathy of diabetic appearance and renal functional and ultrastructural changes compatible with those observed in diabetes developed Repeated investigations however failed to demonstrate obvious disturbances in glucose tolerance

Electroretinographic studies using a special technique which permits the recording of the oscillatory potentials of the electroretinogram have shown that they are often selectively reduced at a very early stage of diabetic retinopathy (Yonemura et al 1962 Sugita et al 1963 Simonsen 1965 Kojima et al 1966 Tassy 1966 Jacobsen et al 1967 Nakajima & Sugimachi 1966) Simonsen (1965) recorded the oscillatory potentials in a series of diabetics who ophthalmoscopically did not show any signs of retinopathy and observed reduced as well as increased oscillatory potentials He believed the electrophysiological disturbances to precede changes in the retina which were not yet visible ophthalmoscopically Fluorescein angiography might have permitted the early diagnosis of retinopathy in those cases

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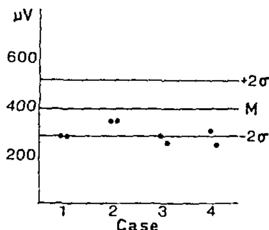


Fig 13

The values of the oscillatory potentials expressed in µV as recorded in the 4 patients presented M mean value in healthy individuals used as controls +2σ and −2σ upper and lower limit of the 95% interval in the controls The values of the oscillatory potentials in the 4 patients investigated lie near the lower limit of the 95% interval of values

their normal values (M 2 σ) The number of cases presented in this paper is too small to permit definite conclusions to be drawn about the appearances of the oscillatory potentials in latent diabetes *i e* whether patients with latent diabetes consistently show changes in the oscillatory potentials

The observations made in the three cases of latent diabetes in which the fasting blood sugar was normal the diabetes remaining obscure until the glucose tolerance test disclosed abnormal values as well as the case of manifest diabetes in which the condition was not recognized until fluorescein angiography revealed the presence of retinopathy strongly suggest that a mild form of diabetic retinopathy may be present without producing any symptoms for quite some time before diabetes is recognized clinically Not until fluorescein angiography indicated the presence of diabetes were the patients referred to the Department of Endocrinology for the glucose tolerance test which showed abnormal values

Owing to its sensitivity fluorescein angiography permits the detection of retinopathy in cases in which ophthalmoscopy alone fails to disclose any abnormality

The use of fluorescein angiography as a routine method for examination of the eye in cases of diabetes of short duration may result in demonstrating that retinopathy develops at an earlier stage than ophthalmoscopy alone has previously suggested

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THEORETICAL ESTIMATE OF THE TEMPERATURE WITHIN IRRADIATED RETINAL VESSELS

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H BEBIE F FANKHAUSER W LOTMAR and A ROULIER

Temperature within irradiated retinal vessels is calculated on the basis of a relatively simple heat transfer model Two mechanisms of heating are considered on the one hand by direct absorption of radiation on the other by conduction from the pigment epithelium which is heated by irradiation The effects of the high pressure xenon arc lamp and the argon ion laser are compared and cooling by blood flow is taken into account Since the irradiation time as used in practical vessel occlusion technique is long compared with the relaxation time of both conduction and convection losses the resulting temperature is roughly that of the steady state Whereas for clear preretinal media it should theoretically be possible to reach temperatures near the boiling point for practically all vessels with the argon laser even at high values of blood velocity such temperatures are obtainable with the xenon arc lamp only for intra and epiretinal vessels Of intravitreal vessels relatively far from the pigment epithelium only big ones with slow flow will respond satisfactorily It should however be noted that vessel occlusion can by no means be predicted in terms of temperature alone

Key words photocoagulation – retinal vessels – temperature calculation – irradiation – occlusion

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In studying the occlusion of pathological retinal vessels by photocoagulation much hope has been placed on the argon ion laser as a light source because of the relatively high absorption of its radiation by blood Preliminary estimates however seem to show that even when a relatively high temperature is reached within a vessel by irradiation this does not necessarily lead to occlusion

A first step towards gaining some insight into the process of vessel occlusion is to calculate temperatures within irradiated vessels This is the aim of the present study

Right at the start it should be pointed out however that occlusion the aim of the treatment is *not a function of temperature alone In contrast to* photocoagulation of the retina (Roulier 1971) practically nothing is known about the effect function relating blood temperature to the production of permanent occlusion of a vessel In addition to temperature this effect function is likely to include many other variables such as time blood velocity profile pressure distribution in the vascular network nature and state of the vessels pressure by edema of the retina generated by the irradiation In other words heat is a *necessary but not sufficient condition of vessel occlusion* as experience has amply shown The reader should therefore be warned that temperatures within vessels as calculated in this study cannot be interpreted without reserve in terms of vessel occlusion Rather it was undertaken with a view to showing how far temperature increase alone can account for the observed facts

When calculating heat effects within retinal vessels we have to consider two mechanisms

(a) direct absorption of radiation by the blood

(b) heating up by conduction from the irradiated pigment epithelium

For both cases we give an estimate of the temperature within the vessels as a function of diameter blood velocity intensity and duration of irradiation length of irradiated part and distance from the pigment epithelium (p e)

The following simplifying assumptions are made

(1) The preretinal media are clear

(2) Irradiation intensity is constant over the cross section of the beam

(3) The absorptance of the vessels and the sensory retina is not changed by the irradiation during the period of exposure

(4) The percentage of red cells within retinal vessels does not differ from that of whole blood

(5) The vessel diameter is not influenced by irradiation

(6) The blood column is moving with constant (mean) velocity v

(7) The numerical values for the specific heat and the heat conductivity of the retina choroid vitreous and blood are those of water
(8) The choroid plays no role in the heat distribution balance (see section 4 d)

The overall temperature increase within a vessel is the sum of the values obtained for both mechanisms Note however that the radiant power absorbed directly by the vessel has to be subtracted from the power of the incident beam in order to obtain that striking the pigment epithelium

In the next section we treat increase of temperature within a vessel by direct absorption on the basis of very simplified assumptions A more sophisticated model is described in section 3 The second mechanism heating up of vessels by conduction from the irradiated pigment epithelium is dealt with in section 4 and results are summarized in the last section

LIST OF SYMBOLS USED

$a_{\parallel}$	fractional ab orption by a parallel layer of blood
a	fractional absorption by a cylinder of blood
a_p	fractional absorption of the pigment epithelium (p e)
$A(x) - \frac{x}{2}\sqrt{1+x} - \frac{x}{-}\ \frac{1}{2}\ln[x+\sqrt{1+x}]$	
b	radius of cylinder around a vessel contributing to heat exchange
c	specific heat of water (4 9 Joule per gram and centigrade)
D	vessel diameter
$d = 1/R^2$	
E	energy
$E(\lambda)$	molecular extinction coefficient of hemoglobin
$erf(z)$	error function
G	Green function
I	intensity of incident beam (Watt/cm²)
L	length of vessel irradiated
L	length of vessel affected by heat from irradiated p e
$m = 4/(\text{thickness of p e})^2$	
N	power of incident beam
N_{bs}	power absorbed
N_{II}	power loss by heat conduction
N_B	power loss by heat convection
$n(x)$	power absorbed per volume
R	radius of irradiated area on p e
$S(\lambda)$	fract onal transmission of preretinal media
T	temperature
T_{∞}	equil brium temperature ($v = 0$)
T_{∞}	equilibrium temperature ($v \neq 0$)

t	time
t_0	characteristic time constant for heating up of a cylinder
v	mean blood velocity within a vessel
V	volume
x	distance from p e
α	extinction coefficient of blood
β	reduction factor taking account of heat losses by convection
γ	density of blood
$\varepsilon(\lambda)$	emission spectrum of light source
η	efficiency factor
λ	wavelength
ϱ	diffusivity (1 5 × 10^{-3} cm^2 sec^{-1} for water)
τ_H	relaxation time of heat loss by conduction
τ_B	relaxation time of heat loss by convection
τ_c	combined relaxation time
τ_E	exposure time
φ	integration variable (integration over cylinder volume)

2 Simple model of temperature increase within irradiated vessels

Mayer & Richey (1964) calculated the temperature distribution in the eye during intense irradiation of small retinal areas We have adopted their symbols and many of the relations they derived

We consider a single vessel in a transparent environment If we call the vessel diameter D the diffusivity ϱ* and assume the blood velocity v to be zero then a temperature increase generated by a short heat pulse will decay immediately after the pulse with a time constant (relaxation time) of the order of

$$\tau_H = \frac{D}{6\varrho} \tag{1}$$

In the case of a long cylinder (length L ≫ D) this is the time it takes for the temperature on its axis to drop to about 30 % of its initial value

If we now assume a finite value v for the blood velocity another time constant τ_B for the temperature decay by *heat convection can be defined in a first* approximation by

$$\tau_B = \frac{L}{v} \tag{2}$$

L being the length of the vessel which has been uniformly heated up by the pulse τ_B is the time it takes for the blood column to move a distance L

* ϱ is the constant in the differential equation $\frac{\delta T}{\delta t} = \varrho \Delta T$ of conduction of heat

If we assume as an upper limit a value of 0 02 cm (200 μm) for the diameter of a pathological vessel to be treated by irradiation it follows from (1) that $\tau_H \leq 0\ 04$ sec Even if there is no blood flow the loss of heat from a vessel will therefore be comparatively rapid when pulse time is of the order of 1 sec

We can estimate the relative importance of these two loss mechanisms by forming their ratio

$$\frac{\tau_H}{\tau_B} \approx \frac{D\ v}{6\ \lambda L} \tag{3}$$

Let us compare two extreme examples In the first we assume a small vessel of 20 μm diameter and a blood velocity of 1 cm/sec The length L heated by irradiation is taken to be 600 μm corresponding to xenon arc coagulation at small field stop We obtain $\tau_H/\tau_B = 0\ 007$ in other words heat loss by lateral conduction (τ_H) is much more rapid than by longitudinal convection

In the second case we assume D = 200 μm v = 5 cm/sec L = 100 μm the latter value corresponding for example to the focus spot of a laser We find $\tau_H/\tau_B = 11$ that is the effect of longitudinal convection is dominant

A quantitative estimate of the temperature generated within the vessel can be obtained as follows

The energy Δ E absorbed by volume element Δ V of a vessel is proportional to the radiation intensity I the linear absorption coefficient of blood α and the exposure time τ_E

$$\Delta E = I\ \alpha\ \tau_E\ \Delta V \tag{4}$$

This is strictly correct only if exponential damping of absorption can be neglected If there were no losses this would result in a temperature increase of

$$T = \frac{\Delta E}{c\gamma\Delta V} = \frac{I\alpha\tau_E}{c\gamma} \tag{5}$$

where T is the temperature above 37 C c is the specific heat and γ the density of blood

In the presence of losses the temperature instead of increasing proportionally to the exposure time will go up slower and slower with time and finally reach asymptotically an equilibrium temperature T_∞ characterized by equality of the rates of absorption and of loss of energy If the exposure time is long compared with the relaxation time of the losses ($\tau_E \gg \tau_H\ \tau_B$ i e $\tau_E > 0\ 5$ sec) the equilibrium temperature T_∞ is to a first approximation

$$T_\infty = T\frac{\tau_c}{\tau_E} \tag{6}$$

where τ is the resultant relaxation time due to the combined losses

From (6) and (5) we find

$$T_{\sim} = \frac{I a \tau_e}{c \gamma} \quad (7)$$

The combination of energy losses may be treated approximately like that of fluid losses by a container with leaks namely

$$\frac{1}{\tau_e} = \frac{1}{\tau_H} + \frac{1}{\tau_B} \quad (8)$$

Introducing τ_e of (8) into (7) and using (1) and (2) we get

$$T_{\sim} = \frac{I a}{c \gamma} \cdot \frac{1}{\frac{6a}{D} + \frac{v}{L}} \quad (9)$$

From this formula we can calculate in principle the temperature within an irradiated vessel We do not however give curves or tables of vessel temperature calculated from (9) for reasons mentioned in the next section

3 Calculation of vessel temperatures on more detailed assumptions

The model used in the last paragraph is rather crude in two respects

a) We have treated heat conduction by means of the characteristic time τ_H For blood velocity $v = 0$ at least it is however possible to apply exact formulas from heat conduction theory

b) We have supposed that *along the ray path energy absorbed per volume* is constant (no exponential decrease) For vessels above 100 μm diameter and the spectral range of strong absorption this is however no longer valid and the exact absorption law should therefore be applied

We proceed to derive formulas taking account of these two points

We consider a cylindrical vessel of diameter D in transparent surrounding which is irradiated by a homogeneous cylindrical beam of diameter L and precorneal power N The beam is assumed to be perpendicular to and centered on the vessel that is the cylinder axes cross at right angles (Fig 1)

We treat first the case L > D The power absorbed

$$N_{ab} = \frac{LD}{\pi (L/2)^2} N a (D) \quad (10)$$

where LD is to a good approximation the cross section of the vessel exposed to the beam $\pi(L/2)^2$ is the total cross section of the beam and a (D) is the fraction of power absorbed by the irradiated volume in terms of the precorneal power of that part of the bundle which hits the vessel a (D) is the product of the source emission the blood absorption and the transmission of the preretinal

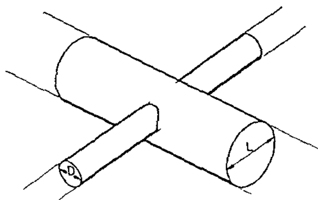


Fig 1
Model of light bundle of diameter L irradiating a vessel of diameter D (L > D)

media integrated over all wavelengths and over the volume (cylinder) involved The integration is done in two steps First we obtain the fractional absorption of a parallel layer of thickness D

$$a_1(D) = \frac{\int d\lambda \;\; \varepsilon(\lambda)\left[1 - e^{-D\,\alpha(\lambda)}\right] S(\lambda)}{\int d\lambda \;\; \varepsilon(\lambda)} \tag{11}$$

$\varepsilon(\lambda)$ is the emission spectrum of the source in arbitrary energy units $\alpha(\lambda)$ is the absorption coefficient of blood and $S(\lambda)$ the percent transmission of the preretinal media

From a_1 the value a (D) for a cylindrical vessel of diameter D can be calculated

$$a\,(D) = \int_0^{90^\circ} d\,(\cos\varphi) \quad a_1\,(D \sin\varphi) \tag{12}$$

In Fig 2 a (D) is plotted versus D for the high pressure xenon lamp and the argon laser At the same time the values of a (D) are given It is seen that they differ from a (D) by a nearly con tant factor of 1 2 On the other hand it is seen that for diameters from 10 to 100 μm the absorption of argon laser light is about 2 4 times that of xenon light

For the calculation of the values shown in Fig 2 the following data were used

$$T_{\infty} = \frac{I a \tau_c}{c\rho} \qquad (7)$$

The combination of energy losses may be treated approximately like that of fluid losses by a container with leaks namely

$$\frac{1}{\tau_c} = \frac{1}{\tau_H} + \frac{1}{\tau_B} \qquad (8)$$

Introducing τ_c of (8) into (7) and using (1) and (2) we get

$$T_{\infty} = \frac{I a}{c\rho} \quad \frac{1}{\frac{6\varrho}{D} + \frac{v}{L}} \qquad (9)$$

From this formula we can calculate in principle the temperature within an irradiated vessel We do not however give curves or tables of vessel temperature calculated from (9) for reasons mentioned in the next section

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where LD is to a good approximation the cross section of the vessel exposed to the beam $\pi(L/2)$ is the total cross section of the beam and $a(D)$ is the fraction of power absorbed by the irradiated volume in terms of the precorneal power of that part of the bundle which hits the vessel $a(D)$ is the product of the source emission the blood absorption and the transmission of the preretinal

as reported by Lemberg & Legge (1949) These authors use units of (mol/liter 1 m 1 for the molecular extinction coefficient E(λ) and base 10 in the absorption law whereas we have used the extinction law in the form I = I exp (−αD) If we assume a hemoglobin concentration in whole blood of 150 g/liter and take the molecular weight to be 16 700 g/mol the relation between α(λ) and E(λ) becomes

$$\alpha(\lambda) = \frac{150 \text{ g/liter}}{16\,700 \text{ g/mol}} \ln 10 \quad E(\lambda) \tag{13}$$

(3) The *transmission values of the human preretinal media* were taken from Geeraets et al (1960) The integrations (11) were carried out numerically by insertion of values for every 10 nm from 380 to 500 nm and for every 20 nm from 500 to 1000 nm

For certain purposes it may be of interest to know the values of a (D) which would result when the absorption of the preretinal media were neglected We have found as a result of the integrations (11) over the spectra that this absorption amounts to 12 % for the xenon and to 27 % for the argon laser source

The calculation of temperature within an irradiated vessel for v = 0 starts again from the premise that the solution for the steady state is a very good approximation By investigation of the well known integral

$$T(x\ t) = \int d^3 x' \int dt'\ G\ (x - x'\ \ t - t')\quad n(x')$$

where n(x) is absorbed power per volume and G is the Green function to the time dependent differential equation of heat conduction in homogeneous media we have found that in a characteristic time interval of the order

$$t_0 \cong \frac{DL}{8a} \tag{14}$$

the temperature at the center of the cylinder rises to about 50 % of the equilibrium temperature For $D \leq 100\ \mu m$ and $L \leq 1$ mm this amounts to 0 08 sec which is much smaller than a mean exposure time of say 0 5 sec So the temperature will very nearly reach the equilibrium value and it is legitimate to apply steady state theory We omit the rather cumbersome details of the investigation mentioned

The basic formula of steady state heat conduction theory is known to be

$$T(x) = \frac{1}{4\pi\varrho c\gamma} \int d^3 x' \ \frac{n(x')}{|x - x'|} \tag{15}$$

where T(x) is the temperature at point x and n(x) the absorbed power per volume at point x By this formula the heat losses through conduction to infinite surroundings are exactly accounted for The integral (15) will not be much affected if we replace n(x) by its spatial mean value

$$\bar{n} = \frac{N_1}{\pi (D/2)^2\ L} \tag{16}$$

(1) *Emission spectra.* Data about the output of the high pressure xenon arc lamp at various loads have been published by Littmann (1962) and Ham et al. (1963). Although the spectral distribution varies somewhat through the load range of 40 to 180 amp which is used on the Zeiss coagulator, it was found that $a_{||}$ (D) is affected only to a few percent. We have therefore chosen a mean value. The argon laser at our disposal (Seelig & Banse 1970) emits, at the level used, mainly two lines of about equal power at 488 and 514 nm. A third group of lines at 476 nm carries less than 10% of the output. Similar values have been reported by L'Esperance (1968) for the laser he used. In the calculation of $a_{||}$ (D) we have neglected this group.

(2) *Absorption of blood.* Absorption spectra of human whole blood have been reported by Bredemeyer et al. (1962). Their results, however, are in serious disagreement with what is known about the spectral extinction coefficient of hemoglobin, the values calculated from their curves being much too low. In order to elucidate this discrepancy we have made a spectrophotometric study of the absorption of whole blood. In the regions of high absorption we obtained values close to those reported for hemoglobin. Since the latter seem to be well established by many investigations, we have used these data for the present paper. The values were taken from the work of Sidwell et al. (1938).

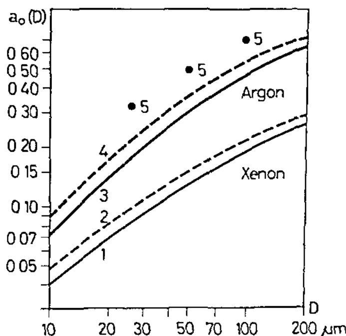


Fig. 2

Fractional absorption by retinal vessels in terms of precorneal power. Absorption of preretinal media is accounted for.
Curves 2 (xenon) and 4 (argon) are for a parallel layer of thickness D, curves 1 (xenon) and 3 (argon) are for a cylinder of circular cross section.
For curves 1 to 4 known absorption values for hemolyzed hemoglobin have been used. Points 5 (argon): for layer of thickness D, but from measurements of whole blood.

$$N_H \cong \frac{T'_\infty}{T_\infty} N_{abs} \tag{19}$$

This equation is analogous to eq (6) From (19) and (17) we have

$$N_H = \frac{\tau o c \gamma L}{A(\kappa)} T'_\infty \tag{20}$$

Losses by convection are

$$N_B = \tau (D/2) \; v\gamma \; c \; T_\infty \tag{21}$$

that is the product of heated mass removed specific heat and temperature Under steady state conditions

$$N_{abs} = N_H + N_B \tag{22}$$

which again is analogous to (8)

If we insert the expressions (10) (20) and (21) into (22) we get an equation for the unknown T_∞ which can be solved

$$T_\infty = \frac{16}{\tau} \frac{N \, a \, (D)}{c\gamma D} \frac{1}{\frac{4o}{A(\kappa)} \left(\frac{L}{D}\right)^2 + vL} \tag{23}$$

Writing this in terms of intensity I within the focus spot of the beam we obtain

$$T_\infty = \frac{4}{\tau} \frac{I \, a \, (D)}{c\gamma D} \frac{1}{\frac{4o}{A(\kappa) D} + \frac{v}{L}} \tag{24}$$

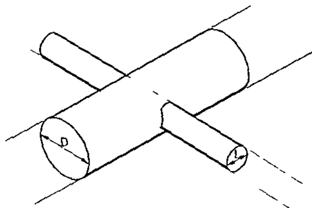


Fig 4
Model of light bundle irradiating a vessel when L < D

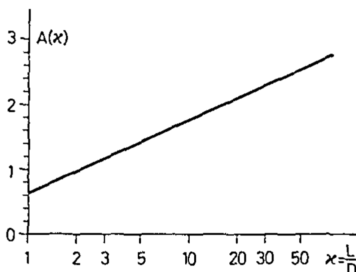


Fig 8
The ancillary function $A(\kappa)$ (Eq 18)

This will cause only a small error since the vessel is irradiated uniformly along its length and on the other hand heat equalization over its cross section is relatively rapid Total power absorbed is not modified

For the center of the cylinder the remaining integration $\int d^3x' \, |x - x'|^{-1}$ over the absorbing cylinder is straightforward and yields

$$T_{\infty} = \frac{N_0}{2\pi c \lambda L} \quad A(\kappa) \tag{17}$$

with

$$A(\kappa) \equiv \frac{\kappa}{2} \sqrt{1+\kappa^2} - \frac{\kappa^2}{2} + \frac{1}{2} \ln\left[\kappa + \sqrt{1+\kappa^2}\right] \tag{18}$$

$$\kappa \equiv \frac{L}{D}$$

The ancillary function $A(\kappa)$ which is a pure number of order 1 and depends only slightly upon κ is shown in Fig 8

We have now still to consider the losses caused by blood flow A somewhat *rough estimate closely analogous to that leading to eq (2) can be made* as follows

Under the equilibrium conditions which we have assumed to prevail and for $v = 0$ the vessel loses the whole of the absorbed energy N_0 to its surroundings If now there is a second source of losses added as in the case $v \neq 0$ the new equilibrium temperature T'_{∞} will be smaller than T_{∞} This again affects the losses by conduction alone which will also be smaller

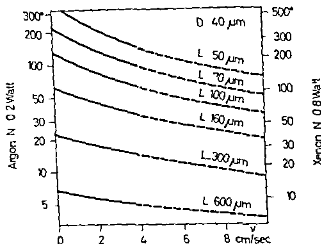


Fig 5c

This expression is exactly of the same form as eq (9) but a is replaced by $4a\,(D)/\gamma D$ and 6ρ by $4\rho/A(\kappa)$ thus accounting for finite values of L/D Note that formulas (23) and (24) are of a high degree of accuracy for $v = 0$ since the only and well founded assumption made is that of steady state whereas the method adopted to allow for the influence of blood flow ($v \neq 0$) is only approximate

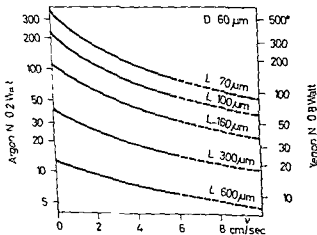


Fig 5d

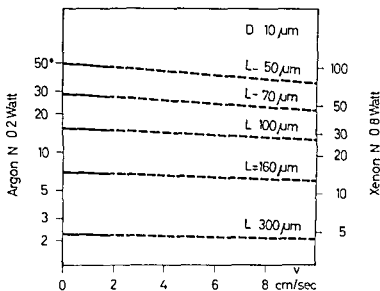


Fig 5a

Temperature increase by direct absorption of radiation within a vessel as a function of vessel diameter D bundle diameter L and blood velocity v Left ordinate scale argon laser power N = 0 2 W Right ordinate scale xenon arc lamp power N = 0 8 W For other power values temperature varies proportionally

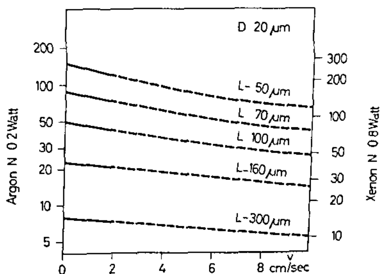


Fig 5b

For a given power of the beam the absorbed power is obviously nearly the same for $L < D$ and $L = D$ In the range $L \leq D$ the temperature at constant beam power will therefore be proportional to $A(D/L)$ If for example $L = D/3$ the temperature is increased by the factor $A(3)$ which from Fig 3 is only about 1 8

Qualitative estimates which we will not reproduce here seem to show that the influence of blood flow ($v \neq 0$) is smaller than in the range $L > D$ and must be taken into account only for big vessels and high velocities ($v\ D > 4\alpha$)

In Figs 5a–f temperatures from (23) are plotted versus v for vessel diameters of 10 to 100 μm and for xenon arc and argon laser light respectively the diameter L being the parameter The results are summarized in section 5

4 Heating of vessels by conduction from the irradiated pigment epithelium

We have seen that heat exchange between a vessel and its surroundings is very rapid compared with exposure time as normally used in vessel occlu ion This is true of course not only for heat generated within the vessel and flowing out to its surroundings but also for the reverse direction In other words the vessel will very rapidly assume the temperature of its immediate surroundings If therefore the pigment epithelium is irradiated it will suffice to calculate the temperature course within the retina and vitreous thus generated as far as heat transfer to the vessel is concerned The cooling effect of blood flow in the vessel on the other hand can be accounted for by a procedure quite similar to that used above

The equilibrium temperatures corresponding to infinite irradiation time as a function of distance from the p e have been calculated earlier (Roulier 1970) Temperatures for finite irradiation time can be derived from these data by introducing an efficiency factor η which will be calculated below

a *Calculation of the equilibrium temperatures* A circular area of the p e (radius R) is irradiated with intensity I (Fig 6) If the spot diameter $L = 2R$ is much greater than the thickness of the p e the equilibrium temperature at a distance x from the p e is given by

$$T(x\ \infty) = \frac{I a_p}{2 c \gamma \varrho} \left(\sqrt{x^2 + R^2} - x\right) \qquad (25)$$

The derivation of this relation from (15) is straightforward a_p is the absorption of the p e as a fraction of the incident precorneal radiation We assume a_p to be constant in time this is true however only for intensities low enough to produce no rapid whitish discoloration of the retina Experience has shown

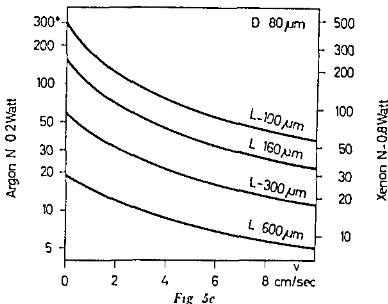


Fig 5e

It remains to consider the case L < D Here too we can approximate the absorbing volume by a cylinder of length D and diameter L (Fig 4) For v = 0 we obtain the temperature at the center of the cylinder by appropriate application of eqs (17) and (18)

$$T_{\infty} = \frac{N_{abs}}{\pi \varrho c \gamma D} A(D/L) \tag{17a}$$

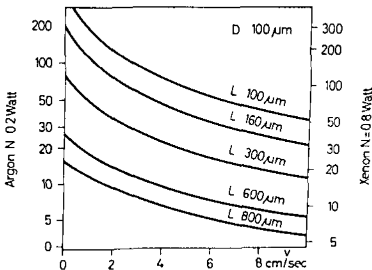


Fig 5f

For a given power of the beam the absorbed power is obviously nearly the same for $L < D$ and $L = D$ In the range $L \leq D$ the temperature at constant beam power will therefore be proportional to $A(D/L)$ If for example $L = D/3$ the temperature is increased by the factor $A(3)$ which from Fig 3 is only about 1 8

Qualitative estimates which we will not reproduce here seem to show that the influence of blood flow ($v \neq 0$) is smaller than in the range $L > D$ and must be taken into account only for big vessels and high velocities (v $D > 4a$)

In Figs 5a–f temperatures from (23) are plotted versus v for vessel diameters of 10 to 100 μm and for xenon arc and argon laser light respectively the diameter L being the parameter The results are summarized in section 5

4 Heating of vessels by conduction from the irradiated pigment epithelium

We have seen that heat exchange between a vessel and its surroundings is very rapid compared with exposure time as normally used in vessel occlu ion This is true of course not only for heat generated within the vessel and flowing out to its surroundings but also for the reverse direction In other words the vessel will very rapidly assume the temperature of its immediate surroundings If therefore the pigment epithelium is irradiated it will suffice to calculate the temperature course within the retina and vitreous thus generated as far as heat transfer to the vessel is concerned The cooling effect of blood flow in the vessel on the other hand can be accounted for by a procedure quite similar to that used above

The equilibrium temperatures corresponding to infinite irradiation time as a function of distance from the p e have been calculated earlier (Roulier 1970) Temperatures for finite irradiation time can be derived from these data by introducing an efficiency factor η which will be calculated below

a *Calculation of the equilibrium temperatures* A circular area of the p e (radius R) is irradiated with intensity I (Fig 6) If the spot diameter $L = 2R$ is much greater than the thickness of the p e the equilibrium temperature at a distance x from the p e is given by

$$T(x \infty) = \frac{I a_p}{2 c \gamma \varrho} (\sqrt{x^2 + R^2} - x) \tag{25}$$

The derivation of this relation from (15) is straightforward a_p *is the absorption of the* p e as a fraction of the incident precorneal radiation We assume a_p to be constant in time this is true however only for intensities low enough to produce no rapid whitish discoloration of the retina Experience has shown

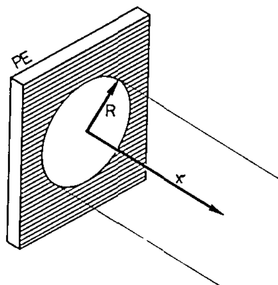


Fig 6
Pigment epithelium irradiated by a cylindrical bundle of radius R

that discoloration cannot usually be avoided either by xenon arc or by argon laser treatment of vessels (Fankhauser et al 1972a b Little et al 1970 Behrendt 1971 L Esperance 1971) Since very little is known about the time constant and degree of this discoloration we have not attempted to take this effect into account The calculated temperatures must therefore be regarded as upper limits

It can be shown that for the assumed model the equilibrium temperature in planes parallel to the p e does not vary more than 20 % over an area the same diameter as the irradiated one (for the sake of brevity we omit the proof) It is therefore a good approximation to assume that a vessel parallel to the p e is embedded in surroundings of constant temperature over this area

b *Efficiency factor* We proceed now to deduce the efficiency factor

$$\eta(x\ t) = \frac{T(x\ t)}{T(x\ \infty)}$$

which has to be applied when the irradiation is of finite duration First we consider the range $x \geq 2R$ Here the irradiated area of the p e can be replaced to a good approximation by a point source of equal total power For this case the solution of the corresponding heat diffusion problem is known to be

$$\eta(x\ t) = 1 - \mathrm{erf}\left(\frac{x}{\sqrt{4\alpha t}}\right) \qquad 0 \leq t \leq \tau_F \qquad (2b)$$

during the interval of irradiation τ_E and

$$\eta(x, t) = \mathrm{erf}\left(\frac{x}{\sqrt{4a(t-\tau_E)}}\right) - \mathrm{erf}\left(\frac{x}{\sqrt{4at}}\right) \qquad t \geq \tau_E \tag{27}$$

for the time after irradiation. The term erf(z) in these equations is the error function

$$\mathrm{erf}(z) = \frac{2}{\sqrt{\pi}}\int_0^z e^{-\xi^2}\, d\xi \tag{28}$$

which is tabulated.

In Figs. 7a, b, c the values of η as a function of time are shown for three values of x, the irradiation time τ_E being the parameter. From these curves it follows that the maximal values for different values of τ_E decrease approximately *inversely as x*.

For small values of x the finite radius of the irradiated area can no longer be neglected. The efficiency factor η for x = 0 has been calculated by Roulier (1970). For an irradiation time of $\tau_E \geq 0.5$ sec, it follows from eq. (13) of that

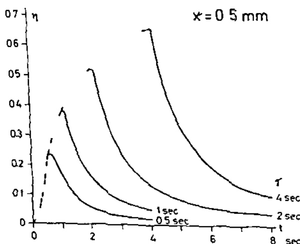


Fig. 7a
The efficiency factor η as a function of time t, exposure time τ_E and distance from the pigment epithelium x

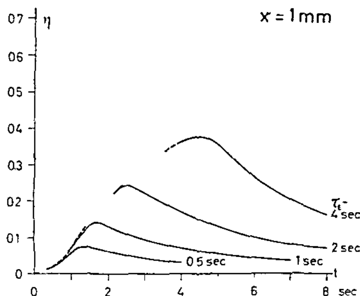


Fig 7 b

paper in good approximation that

$$\eta(0\ t) = \frac{2}{\pi} \operatorname{arc\,tg} \frac{\sqrt{4\varrho t}}{R} \qquad 0\,5 \leq t \leq \tau_E \tag{29}$$

as shown in the annex It may be noted that η reaches its maximum for $t = \tau_E$ that is immediately at the close of irradiation

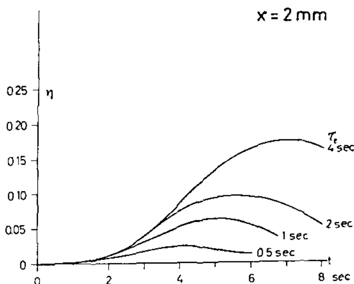


Fig 7 c

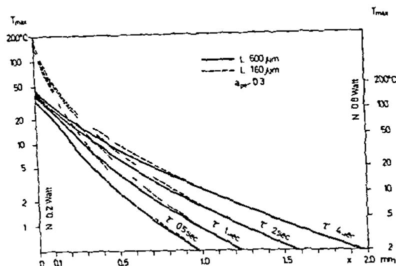


Fig 8

Peak temperatures of vessels heated by conduction from the irradiated pigment epithelium as a function of distance x from the p e exposure time τ_E and beam diameter L for v = 0 Left scale power = 0 2 W right scale power = 0 8 W Influence of the choroid has been neglected Absorption of the p e is assumed to be 0.3 irrespective of the nature of the radiation For other values temperature varies proportionally

In order to obtain a survey of the vessel temperatures induced by absorption in the p e we consider the maximum temperatures which are reached at distance x from the p e for a given exposure time As has been explained above the maximum temperature is the product of the equilibrium temperature T (x ∞) and the maximal value of the efficiency factor η_m (x)

In Fig 8 these maxima of temperature are shown for typical focus spot dimensions of the xenon arc and the argon laser coagulators as used by us (Fankhauser et al 1972a b) For the absorption factor a_p the value of 0 3 was assumed (see Roulier 1970)

Within the range $0 \leq x \leq 2$ R values were obtained by numerical integration of eq (11) of Roulier Temperature scales are given for two different power values of the incident beam namely 0 8 and 0 2 W these values being more or less typical for xenon arc and argon laser coagulation conditions respectively

For equal power of the beams the temperatures at the *surface* of the p e are about 4x higher for the argon laser At the surface of the retina (x = 0 14 mm) this factor has decreased to 2x however and for a distance of over 1 mm from the p e the values are the same which follows of course from the assumptions made

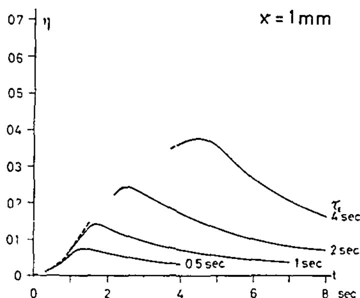


Fig 7 b

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$$\eta(0\ t) = \frac{2}{\pi} \operatorname{arc\,tg} \frac{\sqrt{4\alpha t}}{R} \qquad 0\,5 \leq t \leq \tau_F \tag{29}$$

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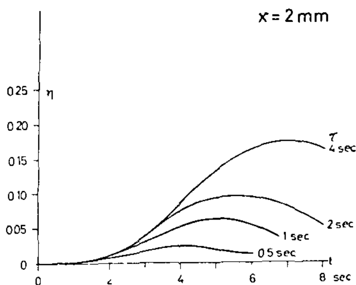


Fig 7 c

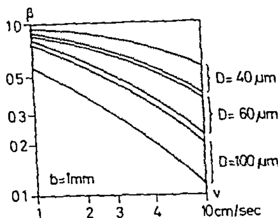


Fig 9

The factor β indicating the fraction of the temp rature (as read from Fig 8) which is reached within a vessel when $v \neq 0$ The three stripes represent vessel diameters D of 40 60 and 100 μm The upper borders of the stripes correspond to L = 600 μm the lower borders to L = 300 μm Radius b in eq (30) has been assumed to be 1 mm

Moreover it should again be remembered that the derivation of eq (32) rests on rather crude assumptions even more so than that of eq (24) for $v \neq 0$ The values calculated from these formulas should therefore be regarded as correct only in order of magnitude

Fig 9 shows values of β versus v for different vessel diameters and the range 300 to 600 μm of L For thin vessels the temperature reduction is relatively small up to high values of v whereas for big vessels it is considerable even for low velocities In this figure only the range $x \lesssim L$ is accounted for

The temperature T within the vessel for finite rates of flow is obtained by multiplying the value of T_m read from Fig 8 by β

An analogous factor β can also be derived from eq (9) We have only to divide the resulting temperature with flow losses included by that without such losses (v = 0) This leads to

$$\beta = \frac{T_{\sim}}{T_{\sim}} = \frac{1}{1 + \frac{D\ v}{6\ L\ \varrho}} = \frac{1}{1 + \frac{\tau_{H}}{\tau_{B}}} \qquad (33)$$

The difference between the two expressions is the factor $^3/_4 \ln(2b/D)$ in the denominator it is a consequence of a somewhat more detailed consideration leading to (32) and of the situations not being exactly the same

c. *Influence of the blood stream.* When the surrounding tissue of a vessel is of higher temperature it is cooled by blood coming from an area of normal temperature and this loss is not compensated immediately from tissue farther away because of the small value of the diffusivity a. In the equilibrium or near equilibrium case which prevails, the vessel will therefore lie across the irradiated area in a "tube" of temperature T lower than the one calculated for flow rate zero, T_{st}. The power loss by convection is given by (21) with T_{st} being replaced by T. In equilibrium, on the other hand, this power is fed by heat conduction from the outer surface of the surrounding "tube" and it remains to estimate the radius b of this tube. Using eq. (1) we can define a characteristic radius

$$b = \sqrt{6 a \tau_i} \tag{30}$$

meaning the distance of heat conduction during exposure time τ_i. With these data we can construct a simplified calculable heat conduction model. At distance b from the vessel axis the temperature is T_{st}, on its surface it is T ($T_{st} > T$). From steady state theory of heat conduction it follows that the power N_f streaming towards the vessel is connected to the temperature difference $T_{st} - T$ and the length L of the "tube" by

$$T_{st} - T = \frac{N_f \ln(2b/D)}{2\pi L\, \rho c_f} \tag{31}$$

when effects at the ends of the heated part of the vessel are neglected. If we introduce the value of N_f from (21) we get for the temperature reduction ratio

$$\mu = \frac{T}{T_{st}} = \frac{1}{1 + \frac{D v}{8 L a} \ln(2b/D)} \tag{32}$$

From this expression it can be seen that the exact choice of b is rather uncritical because only its logarithm appears.

In eq. (21) we have introduced a new symbol L for the following reason. Near the pole ($x \sim 1$) the length of the "tube" contributing to heat flow towards the vessel can roughly be taken to equal the beam diameter L. For greater distances however ($x \sim 1$) we have approximated the irradiated area of the pole by a point source which of course produces a temperature distribution of spherical symmetry. A well founded definition of the "tube" length is therefore hardly possible within this range. We propose to use $L \simeq x$. The consequences of this arbitrariness are mitigated only by the fact that for $x \sim 2$ mm the temperature increase at heating of the pole is almost negligible.

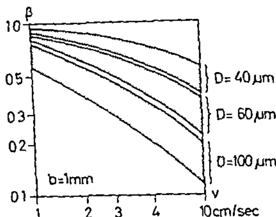


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The difference between the two expressions is the factor $\frac{3}{4}\ln(2b/D)$ in the denominator it is a consequence of a somewhat more detailed consideration leading to (32) and of the situations not being exactly the same

In deriving (32) we have tacitly supposed that during the time interval being studied namely τ_L the temperature at distance b from the vessel T_{max} is constant Although strictly speaking this is of course a contradiction it is nevertheless true that for a time of the order of τ_L the temperature is found to lie between 80 and 100 % of the maximum This can be confirmed on Fig
but holds also for other data within the range in question

d *Role of the choroid* In calculating the heat waves issuing from the p e when it is hit by pulses of radiant energy we have taken no account of the choroid For the rabbit it is however known (Geeraets et al 1962a) that the choroid absorbs about the same percentage of the impinging radiation as the p e

This would lead to roughly twice the temperature values derived from eqs (25) to (32) On the other hand Geeraets et al (1962b) have found that heat convection by the blood flow within the choroid accounts for a 50 % increase in energy required to produce a lesion of given degree as compared with the energy necessary in the absence of flow (dead animal) This factor of 1 5 corresponds to an exposure time of 1 sec and can therefore be applied to vessel treatment The net effect is that absorption and cooling by the choroid roughly compensate each other We are therefore justified in disregarding the influence of the choroid to a first approximation Note however that when the contribution of the choroid to retinal temperature is positive it compensates for the losses by the whitish discoloration produced by coagulation

5 Conclusion

If our calculations can be taken to be at least approximately significant the results would lead to the following conclusions

1 High temperatures within single intravitreal vessels at a relatively great distance from the p e are obtainable with the xenon arc coagulator only for relatively great diameters and very low flow rate With the argon laser on the other hand high temperatures in both big and small vessels even at high rate of flow can easily be achieved

2 Epi and intraretinal vessels of all diameters can also be sufficiently heated up by the xenon source at slow flow via absorption by the pigment epithelium Big vessels with high blood velocity however will not reach high temperature by this mechanism

A discussion on the relations between these results and the effect of vessel occlusion will be given elsewhere

APPENDIX

Derivation of Eq. (29)

Roulier (1970) has calculated the temperature T at the center of the p.e. as a function of the time t elapsed from the start of irradiation on $(0 \leq t \leq \tau_E)$. With his symbols

$$T(0,t) \approx \frac{\kappa a_p}{2 \varrho \sqrt{d}} \left\{ \operatorname{arctg} \sqrt{\frac{d}{m} + 4 d \varrho t} - \sqrt{\frac{d}{m}} \right\} \qquad \text{(A 1)}$$

corresponding to his eq. (13). $m^{-1/2}$ is approximately half the thickness of the p.e. and $d^{-1/2}$ the radius of the irradiated area R. An exact identification with quantities as used in the present paper is not possible because Roulier adopted Gaussian distributions for both the intensity across the beam and the absorption curve of the p.e. Experience shows, however, that the use of Gaussian instead of square *distributions results in errors* of only about 10 %.

$$\text{Since } m^{-\frac{1}{2}} \simeq 5\,\mu\text{m and } d^{-\frac{1}{2}} \simeq R \geq 80\,\mu\text{m}$$

$$\frac{d}{m} \ll 1 \qquad \text{(A 2)}$$

In addition, for $t \geq 0.5$ sec

$$4 d \varrho t \gg 1 \qquad \text{(A 3)}$$

as can easily be verified. With (A2) and (A3) we can approximate (A1) by

$$T(0,t) \simeq \frac{\kappa a_{pe}}{2 \varrho \sqrt{d}} \operatorname{arctg} \sqrt{4 d \varrho t} \qquad \text{(A 4)}$$

and

$$T(0,\infty) \simeq \frac{\kappa a_p}{2 \varrho \sqrt{d}} \cdot \frac{\pi}{2} \qquad \text{(A 5)}$$

from which we obtain eq. (29) of the text by

$$\eta(0,t) = \frac{T(0,t)}{T(0,\infty)} = \frac{2}{\pi} \operatorname{arctg} \sqrt{4 d \varrho t} = \frac{2}{\pi} \operatorname{arctg} \frac{\sqrt{4 \varrho t}}{R} \qquad \text{(A 6)}$$

holding for $0.5 \text{ sec} \leq t \leq \tau_E$

Acknowledgement

We thank Dr. B. H. Crawford for help with the manuscript and critical remarks.

In deriving (32) we have tacitly supposed that during the time interval being studied namely τ_F the temperature at distance b from the vessel T_{max} is constant Although strictly speaking this is of course a contradiction it is nevertheless true that for a time of the order of τ_F the temperature is found to lie between 80 and 100 % of the maximum This can be confirmed on Fig 7 but holds also for other data within the range in question

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STUDIES OF THE GENERATION OF EXPERIMENTAL THROMBOSIS BY ILLUMINATION WITH INTENSE LIGHT

BY

H BEBIE and F FANKHAUSER

The obstruction of glass capillaries perfused with citrated blood by irradiation with intense light has been performed in order to simulate photocoagulation of retinal vessels under greatly simplified conditions Flow velocities were varied between 0 5 and 5 cm sec^{-1} and temperature and change of flow velocity were studied for irradiations which were followed by occlusive and non occlusive events The experiments indicate that irradiation induced temperature rise interferes strongly with blood rheology when blood temperatures of about 60°C are approached For flow velocities below 2 5 cm sec^{-1} the minimum temperature leading to vessel obstruction was found to be 60 C whereas for higher flow velocities this minimum thrombus inducing temperature rises rapidly The rheological events leading to thrombus formation are complex as evidenced by the flow velocity and temperature changes recorded during irradiation

Key words photocoagulation – retinal vessels – photic vessel occlusion

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The obstruction of glass capillaries perfused with citrated blood by ir radiation with intense light has been performed in order to simulate pho tocoagulation of retinal vessels under greatly simplified conditions Flow velocit es were varied between 0 5 and 5 cm sec^{-1} and temperature and change of flow velocity were studied for irrad ations which were followed by occlusive and non occlusive events The experiments indicate that ir radiation induced temperature rise interferes strongly with blood rheology when blood temperat ires of about 60°C are approached For flow veloci ties below 5 cm sec^{-1} the min mum temperature leading to vessel ob struction was found to be 60 C whereas for higher flow velocities this minimum thrombus inducing temj erature rises rapidly The rheological events leading to thrombus formation are complex as evidenced by the flow velocity and temperature chan es recorded during irradiation

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The morphology of the generated thrombi mirrors the complexity of the rheological events producing them In spite of the differences existing between *in vitro* experiments and events occurring in the living organism these experiments suggest that the irradiation induced obstructions are at least partially due to protein denaturation and precipitation processes which critically depend upon temperature and flow velocity of the irradiated blood column

Since the introduction of the xenon arc for the occlusion of pathological retinal vessels by Meyer Schwickerath (1959) this method has found wide acceptance in the treatment of retinal vascular disease Later this method was greatly advanced by the substitution of the argon laser for the xenon arc The irradiation of pathological retinal vessels by radiation emitted from the argon laser is considered at the present time in spite of its shortcomings to be the therapy of choice in the treatment of early proliferative retinopathy Although it is recognised that retinal vessels can be occluded and although the phenomenon of vessel occlusion by the argon beam has been described by a number of authors very little is known about the occlusion mechanism because basic studies are extremely difficult to carry out on the living subject Since no direct approach to the analysis of the mechanism subserving vessel occlusion in the living eye seemed to be feasible indirect methods had to be adopted to advance our understanding So as a first step Bebie et al (1974) calculated the temperature rise to be expected in retinal vessels when irradiated by intense light of given power basing their model on simplified assumptions Since however temperature and temperature induced thrombosis are related in a complex way this paper does not tell us yet the physical conditions required for vessel obstruction We do not know for instance the critical temperature leading to occlusion for a given set of physical conditions The critical temperature required for vessel occlusion cannot be deduced from observation of occlusion events occurring in the living eye since there is considerable uncertainty about the blood flow velocity in normal and pathological retinal vessels Hence we have studied the temperatures required at different flow velocities for actual thermal thrombosis by experimental *in vitro* methods This was achieved by measuring irradiation induced blood temperature rise versus flow velocity in thin glass capillaries

Methods and Calibrations

Citrated fresh human blood (citric acid 4 7 g – sodium citrate 13 2 g – glucose 25 0 g per liter blood) was driven under constant pressure through glass capillaries with diameters ranging from 104 to 142 μm The inner diameters of the capillaries were de

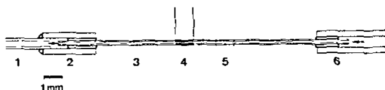


Fig 1

Glass capillary used for irradiation experiments (1) afferent glass tube (2) coupling piece (3) glass capillary (4) platinum winding (5) interception area of irradiation beam with glass capillary (6) coupling piece with afferent blood conducting tube

termined at cross sections near the irradiated area by a precision measuring machine Two diameters at right angles were measured and the mean taken The uncertainty was about ± [illegible] μm Pressure and hence flow velocity were regulated by raising or *lowering the blood reservoir Oxygenated blood was used The degree of oxygenation* varied between 70 % and 90 % but was not determined since this was considered to be irrelevant within the context of these experiments The haemoglobin concentration of the samples of citrated blood varied between 1[illegible] 0 and 15 0 g/100 ml blood

Dispersion of erythrocytes was maintained by a magnetic stirrer in the blood reservoir Imperfect dispersion of the blood was avoided by replacing the blood of the whole system after every irradiation and by control through microscopical examination of the blood conducting capillaries

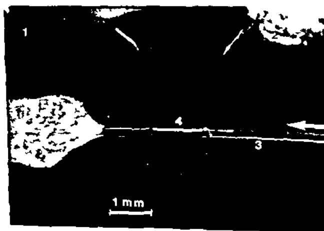


Fig 2

Glass capillary (3) with platinum winding (4) connected to electrodes (1) and (2)

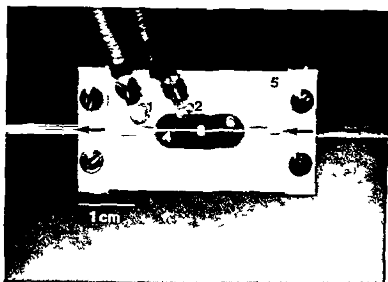


Fig 3
Block of plastic material (5) with mounted capillary (1) and (2) electrodes connected *to platinum winding (4) The illuminated capillary (3) is observed against a black back* ground (6)

Figs 1 2 and 3 show the main elements of the system A tube of 1 15 mm bore was *connected to the capillary via a coupling glass tube of 550 μm bore (Fig 1 (6))* which was screwed to a block of plastic material (Fig 3 (5)) facilitating rapid replacement by a new capillary after an occlusion event had taken place

The blood column flowing within the capillary was irradiated by the beam from a Zeiss xenon arc coagulator equipped with the attachment for coagulation through *the contact glass (Lotmar 1961) The cathode of the xenon high pressure arc and its* mirror image were adjusted so as to result in superposition of the hot spots furnishing an energy distribution as described by one of us (Fankhauser et al 1971 a) which is characterized by an energy peak in the centre of the beam Fig 1 (5) shows the focus of the irradiation bundle impinging on the capillary Downstream from the irradiated *region 30 turns of platinum wire (Fig 1 (4)) thickness 20 μm* were wound tightly round the capillary The right hand end of the platinum windings was maintained accurately at a distance of 1 mm from the centre of the xenon beam The relative posi tions of platinum windings and focus of the xenon beam were controlled through the microscope of the xenon coagulator attachment This microscope (magnification 10×) also served to observe the events occuring in the capillary such as arrest of flow ag gregation phenomena of the erythrocytes and opacification of the plasma proteins The auxiliary illumination provided by the slit lamp which is incorporated in the attach ment proved to be very helpful since it permitted the experimenter to observe the perfused capillaries by direct as well as by darkground illumination The latter was achieved by directing the illuminating beam at high intensity against the plastic mount ing thus illuminating the capillary – which was seen against a black background – from *behind (Fig 3(6)) Still better resolution was obtained by observing the capillary at high* power under a microscope

After havin passed the capillary the blood went through the blood velocity meas uring device (Fig 4) and then left the whole system Irradiation power was measured according to principles outl ned in a previou paper (Fankhauser et al 1971 b) the dosimeter used having been calibrated with a Kipp and Zonen calorimeter

Blood entered the capillary at a temperature of ?5°C (room temperature) The in crease in temperature of the blood induced by irradiation was recorded as a func tion of time by observing the change of the ohmic resistance of the platinum wire described above a constant current was fed into the platinum wire and the voltage drop across it was amplified and recorded on a Beckman dynograph The temperature increase due to this constant current wa negligible Calibration was performed by irrigating the windings with water of known temperature By comparing the voltage variations produced by water of various temperatures forced through the capillary at high velocity with the temperature changes induced by irrigating the windings di rectly it was found that the temperature loss across the walls of the capillary could be neglected and that in fact correct assessment of the temperature of the blood could be expected with this method By moving the irradiating beam downstream to a posi tion just before it touched the platinum windings then moving it back again to its standard position it was also shown that the temperature induced at the site of ir radiation showed only a negligible drop before it reached the platinum windings The temperature change so induced did not exceed ? C Identity of temperature at the irradiation and measuring site however could not be assumed when the blood velocity approached the value 0 and temperature measurem nts obtained after arrest of fluid motion were considered unrealistic

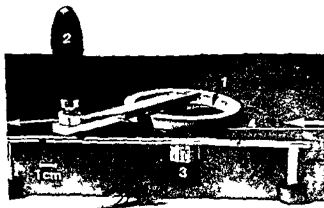


Fig 4

Blood elocity measuring device Pointer (1) is in contact with polyethylene tube de s ibing a loop within a metal ring Crank (?) is connected with pointer (1) and pre cision potenti meter (3) Polyethylene tube (4) is conducting blood towards the meas uring loop

The determination of flow velocity was carried out by following with a pointer the head of the advancing blood column flowing in a polyethylene tube describing a circle of 6 cm diameter within a metal frame (Fig 4) The pointer was connected to a crank the shaft of which was coupled to a precision potentiometer (Fig 4 (3)) The voltage variation across the potentiometer was recorded on the second channel of the dynograph From the distance covered by the blood column thus recorded as a function of time the flow velocities were calculated by differentiating against time taking the actual tube and capillary diameters into account (further details are given below) Within the range of flow velocities in the capillary which were of interest i e 0 5–5 cm sec^{-1} flow velocities in the polyethylene tube whose diameter was about 2 5 times larger than that of the capillary lay between about 0 1 to 1 cm sec^{-1} For these velocities the advancing blood front could be followed with an accuracy of about 0 8 mm Inaccuracies in reading out the position of the blood front from the recordings of the dynograph were of about the same magnitude Taking the ratio of the polyethylene tube and of the glass capillary into account the uncertainty in our knowledge of the distance covered by the blood in the glass capillary was of the order of 0 6 cm The accuracy of the velocities derived by differentiating may be illustrated by the following examples Mean velocities computed from a time interval of 3 sec at a velocity of the order of 3 cm ^{1} will have an accuracy of about , % The inaccuracy of the velocity determination rises by a factor of three if the sampling time is reduced from 3 sec to 1 sec or if the velocity to be measured is only about 1 cm sec ^{1} From this it follows that velocity fluctuations of short duration are suppressed whereas velocities measured after a partial occlusion has taken place or before irradiation has started (i e when there is a long time base of about 10 sec or more for the velocity measurement) will be known with a rather high degree of accuracy It may be concluded that at low flow velocities in particular in the phase of blood arrest the measurement of temperature as well as of blood velocity becomes inaccurate These inaccuracies are however of little importance for the conclusions to be drawn from these experiments as will be realized later

Results

A total of 87 irradiation experiments were conducted using seven capillaries After each irradiation flow resistance was tested and unless partial or total occlusion had been produced was found to be normal At the end of a series of non occluding irradiation events the occlusion threshold was approached by raising blood temperature in steps at various blood velocities until partial or total occlusion occurred (Fig 6) A total of seven occlusion events was recorded together with 74 non occlusion events The average exposure duration chosen (i e the time during which blood temperature was increased) was about 20 sec except for the occlusion events The exposure times required for occlusion are given in Table 1 Fig 5a displays the tracings of a non occlusive irradiation event The upper curve displays the temperature increase above 25 C room temperature (to be read from right to left) while the dis

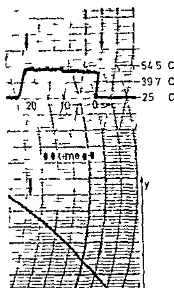


Fig 5a
Example of non occluding irradiation experiment Above temperature rise below distance covered by irradiated blood column y is a linear measure of distance (see text)

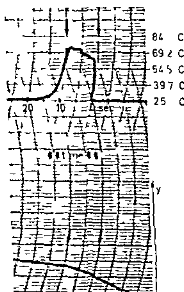


Fig 5b
Example of partial occluding experiment Designation as in Fig 5a (for details see text)

tance covered by the blood column in relative units is shown below. y is a linear measure of the distance traversed by the blood within the capillary. The y values were read into a computer and differentiated against time. For proper normalisation the ratio of the diameters of the capillary and the tube in the tracking device as well as the linear relation between the position of the precision potentiometer and the ordinate had to be taken into account.

In the example given in Fig. 5a it was seen that while the temperature increased from 47 to 51° C flow velocity dropped from 1.6 to 1.4 cm sec^{-1} (pulse duration 24 sec). Fig. 5b shows the tracing of a partial occluding event. On close inspection of the tracing the following details are observed: flow velocity checked during 10 sec before the start of the impulse was found to be 0.8 cm sec^{-1}. When the impulse was started the temperature rose rapidly to 63°C and then climbed irregularly up to 73° C at which point flow velocity suddenly dropped to an apparent velocity of 0.2 cm sec^{-1}. Since at this time a thrombosis had already partially obstructed the lumen of the capillary and because the actual diameter of the partially occluded capillary was not known the true flow velocity was not known either. It is still correct, however, to state that the volume flow rate had decreased to ¹/₄ of its initial value.

It may be noted that due to the disturbance of blood flow the temperature decay time *was more than 5 times longer (Fig. 5b) as compared with the* non-occlusive events (Figs. 5a and 7b).

The results of the irradiation events are summarized in Fig. 6. This Figure provides a survey of the blood temperature* and blood velocities at which our observations were made. Out of the total of 81 irradiation events only those have been incorporated in Fig. 6 in which temperature exceeded 30° C and velocity stood below 5 cm sec^{-1}. The partial and total occlusion events are given without exception. Every solid circle in Fig. 6 symbolizes an irradiation which did not lead to a partial or total occlusion, whereas the arrows and open circles symbolize events which did lead to a partial or total occlusion, the open circles symbolizing starting temperature and velocity, the points of the arrows the temperature and velocity recorded at the end of irradiation.

The shapes of the arrows are a qualitative representation of the change of temperature and blood velocity during the pulse duration. Regarding the accuracy of the arrows the following statement should be made: starting temperature and starting blood velocity are accurately known as is the final flow velocity, whereas the final temperature is poorly defined due to complete or incomplete blood stoppage.

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* Throughout this paper "temperature" is given in degrees above 0° C, whereas "temperature increase" is given in degrees above 25°C room temperature.

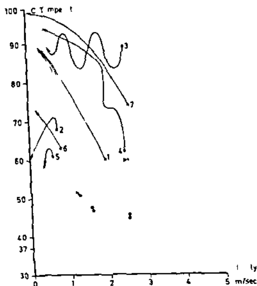


Fig 6

Display of blood temperature – blood velocity combinations leading to occluding (open circles and arrows) and non occluding irradiation events (solid circles) The shaded area divides the two regions (for details see text)

The solid circles of the plot refer to the starting temperatures and velocities reached 1 sec after the impulse had been triggered Due to time delays at various levels it was only at 1 sec after the irradiation pulse had been initiated that the actual temperature level was reached It was observed that flow velocities for non occluding events fell by about 10–20 % during one impulse whereas temperature increased by the same amount indicating constancy of absorbed power From the law of Hagen Poiseuille it may be shown that the increasing friction as the blood advances in the blood velocity measuring tube (Fig 4) is roughly compatible with this deceleration effect In six events for unknown reasons flow velocity showed a tendency to increase by about 10 % during irradiation It has to be remembered in this connection that flow velocity is very sensitive to disturbances such as undetected minute air bubbles adhering to the inner walls of the tube Until a temperature region of about 40 C was reached blood deceleration was roughly in accord with the expected friction increase in the measuring loop Assuming plasma protein denaturation phenomena to start with the denaturation of fibrinogen at about 47 C (Shultz & Knobloch 1955 Burstein & Cuinand 1954) denatured proteins which

may have been produced beyond 45°C could not be shown to have influenced flow velocity significantly. This is not to say that viscosity changes did not occur within this range but rather that they did not show up in this rather crude experimental set up and could not be separated from other effects.

Since the absorbed power is proportional to flow velocity v and temperature increase ΔT ($T = 25\,C + \Delta T$) the product $v \cdot \Delta T$ was calculated during the course of each irradiation event. Deviations from constancy are to be considered indicative of change of power absorption. Absorption varied for unknown reasons from one irradiation event to the next, sometimes by as much as 20%. Since we are interested in temperature levels directly rather than in the required powers, these fluctuations of absorption are of no relevance for the conclusions to be drawn from these investigations. The power levels necessary to induce defined temperatures ranged from 100–800 mW. It should be stressed that there is no basis for comparison with irradiation levels required to induce the same temperature effects in the living eye, because obviously the two situations are radically different (retinal vessel surrounded by water, glass capillary surrounded by air). Therefore irradiation powers are of no relevance in this context and are not given in Fig 6. The question of prime interest, viz. the relation between flow velocity and temperature leading to partial or total occlusion, is elucidated by inspecting the temperature levels which lead to stoppage of blood flow. As Fig 6 shows, the initial temperatures which finally led to obstruction of the capillary are situated above the shaded area. This broad dividing region suggests that for flow velocities below about 2.2 cm sec^{-1} the precondition for capillary obstruction is a minimum starting temperature of about 58 to 62°C, whereas this temperature possibly rises abruptly for higher velocities and may reach a value as high as 72 C for velocities of about 2.6 cm sec^{-1}. It is evident from Fig 6 that due to the paucity of our measuring points, the temperature levels quoted leading to total or partial occlusions cannot be regarded as true threshold temperatures separating two temperature flow velocity fields ending in occlusion or not. The points of Fig 6 leading to occlusion (open circles) may be regarded as upper limits of the threshold. For flow velocities higher than 2.6 cm sec^{-1} our experimental set up proved to be inadequate for inducing occlusive events. Table 1 summarizes the starting and final temperatures of every occluding or partially occluding event together with the pulse durations required for partial or total thrombosis.

It is seen that the time span from the start of irradiation to vessel occlusion varied considerably from 5 to 16 sec, which is not surprising since the blocking mechanism cannot possibly be the same when a complex system such as blood moving at various velocities and heated up to various temperature lev

Table 1

	Number of occluding event						
	1	2	3	4	5	6	7
Temperature (°C)							
Beginning	60	68	89	69	61	63	74
End	89	60	89	93	58	73	99
Pulse duration required for blood occlusion (seconds)	14	5	7	16	13	9	8
Type of occlusion	partial	total	phase of partial occlusion followed by total occlusion	partial	partial	partial	total

els is transformed into an obstructing coagulate It is also evident that the velocity temperature relationships were radically different for the various occlusion events

Microscopical observations related to heat-induced thrombosis

Microscopical observations during irradiation did not show any peculiarities below the shaded area of Fig 6 The erythrocytes were visible only when they did not move otherwise the blood column appeared homogeneous When the shaded region of Fig 6 was approached heterogeneities within the lumen of the capillaries started to appear These heterogeneities originated at the region of irradiation (Fig 1 (5)) and often appeared as a granular pattern Blood fractions containing these heterogeneities were usually followed by fractions which appeared completely homogeneous in alternation As temperature increased and flow velocity decreased the phases during which such heterogeneities were observed increased During irradiations leading finally to thrombosis they were usually but not regularly observed the density of the granulation increased and became more prominent as the occlusion event was approached

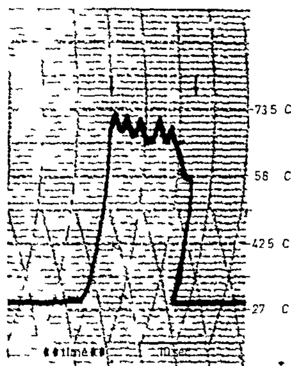


Fig 7a
Non occluding irradiation experiment Strong temperature fluctuations ascribed to microemboli disturbing blood flow

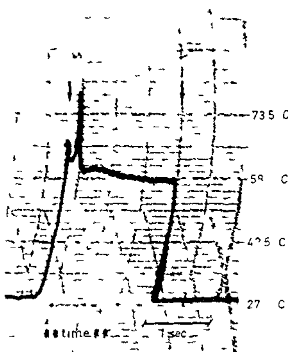


Fig b
Two temperature peaks of different heights due to momentary blood flow interruptions ascribed to microthrombi which were washed away

As seen in Fig 6b irradiation induced blood stoppage is a very rapid process obviously because positive feedback between increasing obstruction decreasing flow velocity and increasing temperature rise comes into play leading to an accelerated rate of change of these quantities A further temperature increase was avoided by an early termination of the pulse as soon as a sudden velocity decrease was observed Because of this rapid time course the component phases of thrombus formation could not be resolved by microscopical observation and in only two irradiation events was it possible to demonstrate clearly the events immediately preceding thrombosis and to record the large rheological disturbances characterizing the prethrombotic phase (Figs 7a and 7b) In the experiment recorded in Fig 7a pronounced heterogeneities of the blood column were observed resulting in large temperature fluctuations In Fig 7b a sudden precipitation of protein on the capillary wall was observed leading to two temperature peaks the higher at 16°C above the slowly rising equilibrium temperature The precipitates were washed away completely when the pulse was interrupted In fact this capillary was found to be entirely clear at the end of the experiment This irradiation event could not be classified under either the occlusive or the non occlusive events It must be concluded then that flow velocities of 3 2 cm sec^{-1} (Fig 7a) to 4 2 cm sec^{-1} (Fig 7b) at the induced temperature level successfully counteract the thrombogenic mechanisms (The terms occlusive and partially occlusive events were by definition reserved for irradiation events in which flow had come down suddenly and permanently to 0 or to a fraction of the initial value and in which microscopical inspection revealed thrombotic material within the capillary) The low velocity blood flow characterizing the partially occlusive events while it could be recorded quantitatively with the flow velocity measuring device could be observed only with great difficulty by observation of the capillary at low magnification (10×) with the slit lamp microscope attached to the photocoagulator In the partial occluding event 6 (Fig 5b) persistence of residual flow escaped slit lamp observation completely

Clearly the occurrence of partial occlusions was the consequence of our irradiation strategy in which irradiation was interrupted at the earliest observable sign of blood stoppage

An examination of the capillaries under high magnification revealed that thrombosis nearly always started from the capillary wall Once total occlusion had occurred the obstruction was strong enough to resist a pressure of 6 atmospheres The first sign of clotting was observed at the centre of the irradiating beam (near the capillary wall) spreading from this point over a variable distance downstream

Several patterns of heat induced thrombi were observed The most com

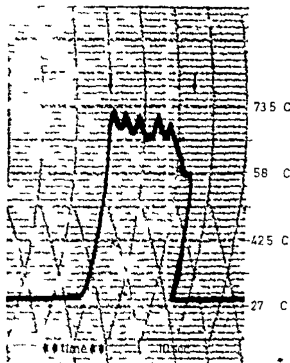


Fig 7a

Non occluding irradiation experiment Strong temperature fluctuations ascribed to microemboli disturbing blood flow

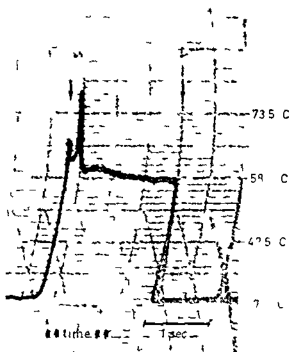
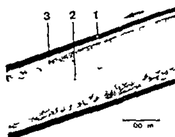


Fig 7b

Two temperature peaks of different heights due to momentary blood flow interruptions ascribed to microthrombi which were washed away



Fi 9
I artial occluding irradiation event (1) wall of glass capillary (°) central fluid blood masses (3) clotted blood adhering to the walls of the capillary

Fig 9 shows a partial occlusion This Figure adds further proof of the as sumption of commencement of coagulation close to the capillary wall Here irradiation was stopped at the first indication of a diminution of flow ve locity In spite of this immediate cessation of irradiation flow velocity fell to a small fraction of its original value although not to zero Observation under the microscope showed that the central part of the capillary still con tained fluid blood which could be moved back and forth by pressure and suc tion

Figs 10a b and c display a more complex configuration of the area occupied by the thrombus as compared with Figs 8a and 8b Whereas Figs 8a and 8b seem to be typical for occlusions reached at low flow velocities (occlusion num bers _ ɔ and 6 of Fig 6) Figs 10a 10b and 10c seem to characterize thrombus

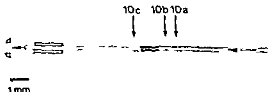


Fig 10
S h ati f gure of capillary The arrows indicate the centres of photographs 10a 10b and 10c.

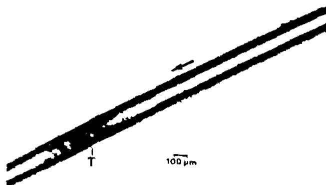


Fig 5a
Simple type of thrombus (T) Fluid blood has been washed out Observe conical shap of empty space upstream from thrombus because of thrombotic masses adhering to th wall of the capillary

mon types are illustrated in Figs 5a and 5b In Fig 5a the fluid blood ha been washed out from both sides of the thrombus (T) Fig 5b illustrates th same type of thrombus configuration before the fluid blood has been removed Both Figures show that the clear central part of the capillary where no clot ting had yet taken place had a conical configuration which suggests that th coagulation process had started from the capillary wall

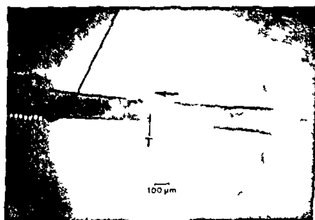
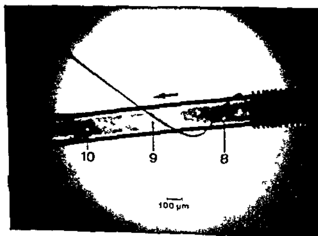


Fig 5b
Simple type of thrombus (T) Fluid blood column narrowing towards the thrombus be cause of thrombotic masses at the walls of the capillary

centres of the subsequent Figs 10a b and c along the capillary In Fig 10a aggregated blood is seen narrowing the lumen upstream (Fig 10a (1)) which embraced an optically empty space (Fig 10a (2)) being followed by two thrombi (Fig 10a (3) and 10a (4)) obstructing the lumen of the capillary (We do not know whether the optically empty space was empty or contained coagulated plasma protein) This was followed further downstream by a looser conglomeration of cellular elements (Fig 10a (5)) Still further downstream the lumen of the capillary was traversed by an irregular column of coagulated protein surrounded by an optically empty space containing only very few cellular elements (Fig 10b (6)) This region again was followed by a zone (Fig 10b (7)) resembling very much that of Fig 10b (5) Below the platinum windings the obstruction was due to aggregated erythrocytes (Fig 10c (8)) and a coagulated protein clot (Fig 10c (9)) which contained only very few cellular elements The erythrocytes (Fig 10c (10)) although they showed signs of aggregation were freely movable by suction and pressure up to the protein clot which seemed to be the chief obstruction to the flow downstream below the platinum windings

Fig 11 which is taken from occlusion number 3 shows a protein clot containing only very little debris of cellular elements The central clear canal (Fig 11 (3)) seems to end at point Fig 11 (2) Although due to limitation of the available material no systematic description of the thrombi produced by irradiation is possible the following general statements seem justifiable the



Fi 10c
Glass capillary at position 10c of Fig 10 Lower end of complex thrombus consisting of elements (8) to (10) (for details see text)

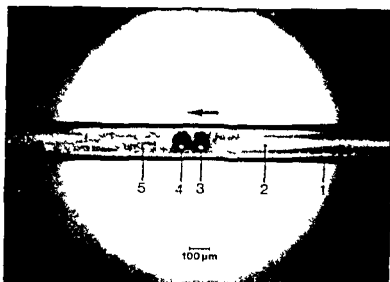


Fig 10a
Glass capillary at position 10a of Fig 10 Upper end of complex thrombus consisting of elements (1) to (5) (for details see text)

formation at higher flow velocities and display the occlusion number 7 The essential characteristic was a much larger spread of the thrombus along the capillary Looking at the capillary under slit lamp magnification the occluded part appeared segmented The schematic Fig 10 indicates the positions of the

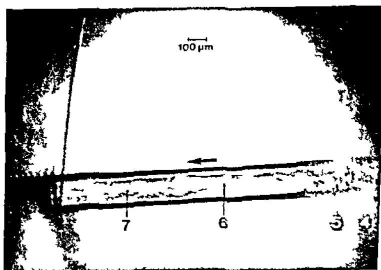


Fig 10b
Glass capillary at position 10b of Fig 10 Central part of complex thrombus consisting of elements (4) to (7) (for details see text)

the clotting mechanisms were not identical in each obstruction event This was well supported by the observation that the end product of occlusion the thrombus also showed marked morphological differences for each obstruction It is obvious that different rheological velocity temperature sequences will in general lead to different end products

The *in vitro* results obtained in these experiments with citrated blood are inadequate to explain the phenomena observed during photocoagulation of vessels in living human or animal eyes However certain speculations seem to be justifiable The current technique of applying multiple pulses of short duration by the argon laser to pathological retinal vessels adjusting the beam diameter approximately to the vessel diameter which was introduced originally by Patz and Zweng et al (1972) may depend on summation of the effects by the pulses delivered in succession As a first hypothesis one may invoke for this photocoagulation strategy a damage mechanism similar to the one induced by other energy forms in particular ruby laser irradiation (Kochen et al 1965) weak electric currents which are able to produce micro lesions in the endoth lium (Frost et al 1969 Honour et al 1971) can induce thrombosis when applied repetitively Such microlesions may not be observed during biomicroscopical observation of the irradiated retinal vessels but may still eventually induce thrombosis by clotting mechanisms such as are shown by these authors The goal of Patz s and Zweng s method and its modifications however is to drive the irradiated retinal vessels by suitably adju ting pulse duration and pulse power into spasm In addition other biomicroscopical phenomena have been observed in the preocclusive phase Bowbyes et al have observed the following phenomena to occur during irradiation of a vessel with the argon beam as the channel narrows a) segmented or granular flow b) darkening of the blood column c) formation of microemboli and their disappearance distally

The authors stress that none of these phenomena are constant Our biomicroscopical observations of the human eye are in as close agreement with these observations as far as is possible with observations which are at or beyond the limit of resolution One may conclude from this that in addition to the microlesion mechanism as postulated above other processes similar to those observed by us in the *in vitro* experiments may be assumed to occur in the living eye The heterogeneities of variable density observed in our *in vitro* experiments may be of the same nature as the phenomena observed by Bowbyes et al and may be regarded as denaturated and precipitated plasma fractions including cellular elements floating as microemboli of variable size in the still fluid plasma The spasms of retinal vessels may be interpreted as the precipitates of plasma proteins at the capillary walls It is highly un

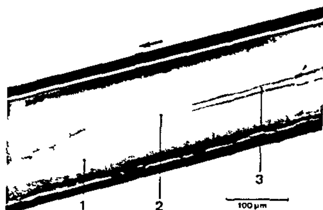


Fig 11
Thrombus consisting of proteinous masses (1) obstructing the capillary Central patent canal (3) ending at (2)

configuration of the thrombi varied from very simple (Figs 8a and 8b) to very complex (Figs 10a 10b and 10c) The simple types (and also Fig 11) suggest that growth of the thrombotic process starts from the capillary wall and proceeds towards the centre of the capillary In addition to mixed corpuscular protein thrombi clots of coagulated protein containing only sparse cellular inclusions were also observed (Fig 11) Here too as noted above the clotting seems to have progressed from the walls towards the centre

In this respect a close analogy seems to exist between the *in vitro* situation and the thrombogenic events occurring in the living eye

Conclusions

As the most prominent result these investigations showed that it is possible to produce heat generated thrombi in glass capillaries of about 100 µm diameter perfused by citrated blood at flow velocities varying from 0.5 to 2.6 cm sec^{-1} when irradiated by intense light – such as is used when photocoagulating retinal vessels – at initial blood temperatures ranging from 60 to 73 C The time required to induce thrombosis varied from 5 to 16 sec The starting temperature required for successful obstruction was found to be about 60 C at a flow velocity of 0.8 cm sec^{-1} and lower than 73 C at 2.6 cm sec^{-1} The different shapes of the flow velocity temperature correlation curves before blood stoppage was completely achieved – as exhibited in Fig 6 – suggest that

Table 9

Flow velocity Temp to be reached	2 0 cm sec-1 60 C		9 6 cm sec-1 75°C	
Beam source	Xenon	Argon	Xenon	Argon
Diameter of focus spot	600 μm	160 μm	600 μm	160 μm
Vessel diameter	100 μm	100 μm	100 μm	100 μm
Beam power required	1 0 W	60 mW	1 6 W	130 mW

9 cm sec-1 and a temperature of 70 C at v = 2 6 cm sec-1 for a Xenon beam with a diameter of L = 600 μm and an Argon beam with a diameter of 160 μm as derived from the graphs in Bebie et al (1974) these beam powers refer to a vessel of 100 μm surrounded by water

The figures for the beam power required do not seem at all unreasonable however this computation should not be taken too literally for several reasons

1 We do not know to what extent the occlusion mechanisms in the living organism are comparable with the mechanisms observed in glass capillaries

The results of Bebie et al (1974) are based on estimates rather than an exact analysis this remark applies especially to the velocity dependence of the temperatures reached in an irradiated vessel

3 The absorption of whole blood is not yet sufficiently known Bebie s numerical results are based on the well known absorption spectra of haemolized blood power absorbed by whole blood may exceed these values

In spite of these difficulties a tentative comparison of the results of Bebie et al and the experiments in glass capillaries with the required beam powers as obtained from clinical experience will be given in a forthcoming paper (Fankhauser et al)

Acknowledgments

The helpful advice of Dr W Lotmar throughout this work is highly appreciated The authors would like to thank Dr E Korber of the Blutspendezentrum der Stadt Bern for supply of fresh human blood for these experiments The glass capillaries were made by E Lenggenhager The difficult and painstaking work of winding the 20 μm

likely that pathological vessels such as are observed in diabetic retinopathy would be able to be thrown into spasm and judging from our experiments a more likely explanation for these biomicroscopically observed "spasms" is the appearance of protein precipitates such as are shown in Fig 11 containing only few cellular elements Segmentation as observed *in vitro* (Figs 10a b and c) may have its counterpart in the segmentation observed with biomicroscopical methods in the living eye

It may be argued that the thrombotic events *occurring in photocoagulation* of the vessels in the living eye may be similar to the clotting events induced in our experiments in so far as they could be explained at least partly as rapid protein denaturation and precipitation – as well as rapid cellular aggregation – phenomena being characterized by specific adhesive properties which cannot be deduced from our experiments This does not exclude the possibility that thrombotic events which typically follow microlesions in the living organism which involve part of the enzymatic cascade would also play an important role In addition our experiments suggest that the apparent interruption of the blood column as seen by slit lamp observation of the glass capillary may not mean that a total occlusion has been achieved and it has been shown that a minimal flow may well persist which may reopen the vessel of the living eye after a variable time delay an occurrence which is in fact often observed when photocoagulating retinal vessels Our experiments suggest then that it may be desirable to prolong a pulse when the first sign of spasm or interruption of the blood column appears although we are aware that this would be associated with an increase in the damage to the surrounding retina Our experiments suggest in addition that slit lamp observation of the irradiated blood column cannot give a reliable indication of the presence or absence of residual blood flow This is in analogy to the living eye (Apple et al 1975) where fluorescein angiography is considered indispensable *in order to reach a decision whether complete blood stoppage has* been achieved or not (Goldberg et al 1973)

It seems worthwhile to attempt a comparison of our experimental results with the predictions of a previous paper (Bebie et al 1974) Here on purely experimental grounds a critical temperature of the order of 60 C was obtained for velocities up to 2 cm sec⁻¹ (about 65–75 C for a velocity of 2 6 cm sec⁻¹ respectively) on the other hand in Bebie et al (1974) temperature in a retinal vessel i e a tube surrounded by water was derived in terms of beam power N beam diameter L vessel diameter D and flow velocity v (see Fig 5f of Bebie et al 1974 for D = 100 µm e g note that temperature in this reference is to be understood as temperature increase above 37 C) Table 2 summarizes the beam power necessary to obtain a temperature of 60 C at v =

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platinum wire on the capillary was done by P Munger by permission of Dr A Perlstain Director of the Swiss National Bureau of Standards Wabern The drawings were made by P Schneider and W Hess The authors are greatly indebted to Prof E A Beck and Drs M Furlan and J P Barras for constructive and helpful discussions We thank Dr B H Crawford for help with the manuscript and critical remarks

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Optic and drug inhibition of the fixing eye and favouring of the amblyopic eye is no novelty During recent years these methods have had a renaissance as th y may under certain circumstances be extremely advantageous and ef fective when used in varying and individual combinations for a long time

These therapeutic methods have recently been widely known as penalization (= punishment or inhibition) which may be carried out in a number of dif ferent modifications The first trials of the so called penalization methods were by Pfandl (1958) and Pouliquen (1958) Among the most recent publications should be mentioned Cuppers (1970) Denys (1971) Quere (1972) Catros & Carrec (1972) Pouliquen (1972) and Weiss (1972)

The penalization methods are said to be effective not merely in squint am blyopia According to some authors this treatment is able to reduce the squint angle rendering the eye position parallel or cosmetically satisfactory without operation in about three quarters of all patients with esotropia (Rethy & Gal 1968 Pouliquen 1972) In addition it has been reported also that the methods can normalize an abnormal retinal correspondence and cure squint amblyopia in cases where conventional occlusion therapy affords no result (Quere 1972)

The therapeutic principles are to inhibit the fixing eye by daily instillation of atro pine and when indicated also by an optic over or under correction During the maintenance phase optic inhibition of the fixing eye is sometimes the only treatment The amblyopic eye is favoured in relation to the inhibited eye by being provided with normal correction or over correction for near The treatm nt is long lasting as the m thods often have to be employed for a year or two combined individually and mo dified gradually as the condition improves

In our Clinic conventional occlusion is still by far the most effective method for treating squint amblyopia It is widely accepted and carried through by patients parents and ophthalmologists so it is exceptional for occlusion the rapy to be given up Among approx 00 squinting children referred to the Clinic in the course of one year there were only about 25 in whom conven tional correct occlusion therapy proved impossible to carry through because of non acceptance by the patient or parents Thus our experience to a marked extent agrees with that of Bruckner & Stamm (19 2) but differs from expe rience in France for example where according to some authors correct oc clusion therapy is impossible to carry through in about 10 % of the patients (Quére 19 ?) Thus clearly the requirement for the penalization methods is far greater in France than in Denmark.

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OPTIC AND DRUG PENALIZATION AND FAVOURING IN THE TREATMENT OF SQUINT AMBLYOPIA

BY

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The authors report the experience of so called penalization methods (optic and drug inhibition and favouring) in the treatment of squint amblyopia in a material of 23 children who had proved resistant to conventional occlusion therapy of a mean duration of 8 months The ineffective occlusion therapy had been performed continuously in three and periodically in 20 of the patients The mean age of the patients was 5 years and the mean duration of squinting was 4 years before penalization therapy was instituted

The unsuccessful occlusion was done by adhesive tape and in the majority *of cases was given up by the patients and parents in only three cases was* it the doctor or orthoptist who decided to abandon the procedure In our Clinic occlusion therapy is rarely given up by the patient or the parents so the present group represents exceptional cases

In 17 of the 23 patients a considerable visual gain was obtained the mean visual acuity in the amblyopic eye increasing from 6/24 to > 6/9 Visual improvement was not obtained in patients in whom conventional occlusion therapy had been carried out correctly and permanently for a long time before being given up The penalization therapy lasted for an average of 10 months and was easy to carry through (only three patients gave up)

Key words squint amblyopia – optic and drug therapy – penalization methods – patients refusal of further occlusion

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Optic and drug inhibition of the fixing eye and favouring of the amblyopic eye is no novelty During recent years these methods have had a renaissance as they may under certain circumstances be extremely advantageous and effective when used in varying and individual combinations for a long time

These therapeutic methods have recently been widely known as penalization (= punishment or inhibition) which may be carried out in a number of different modifications The first trials of the so called penalization methods were by Pfandl (1958) and Pouliquen (1958) Among the most recent publications should be mentioned Cuppers (1970) Denys (1971) Quere (1972) Catros & Garrec (1970) Pouliquen (1972) and Weiss (1972)

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The therapeutic principles are to inhibit the fixing eye by daily instillation of atropine and when indicated also by an optic over or under correction During the maintenance phase optic inhibition of the fixing eye is sometimes the only treatment The amblyopic eye is favoured in relation to the inhibited eye by being provided with normal correction or over correction for near The treatment is long lasting as the methods often have to be employed for a year or two combined individually and modified gradually as the condition improves

In our Clinic conventional occlusion is still by far the most effective method for treating squint amblyopia It is widely accepted and carried through by patients parents and ophthalmologists so it is exceptional for occlusion therapy to be given up Among approx 700 squinting children referred to the Clinic in the course of one year there were only about 25 in whom conventional correct occlusion therapy proved impossible to carry through because of non acceptance by the patient or parents Thus our experience to a marked extent agrees with that of Bruckner & Stamm (1972) but differs from experience in France for example where according to some authors correct occlusion therapy is impossible to carry through in about 0% of the patients (Quere 19[illegible]) Thus clearly the requirement for the penalization methods is far greater in France than in Denmark

Material

The material is strictly selected consisting of 23 children with squint amblyopia in whom conventional occlusion therapy could not be carried through As already mentioned this is uncommon in our Clinic The patients had been treated for an average of 8 months (from 1–24 months) by conventional occlusion with adhesive tape (periodically in 20 cases and continuously in three) whereupon the parents and patients refused to continue In a few cases (three in all) however the occlusion therapy was discontinued at the suggestion of the Clinic because the amblyopia appeared to be refractory to consistently and correctly performed long lasting occlusion therapy

At the conclusion of the unsuccessful occlusion therapy and at the institution of penalization therapy the patients mean age was 5 years 3 and 8 years being the outside limits At that time the patients had been squinting for an average of 4 years

The mean amblyopia in the squinting eye was 6/24 and the material must be considered a heavy one in terms of amblyopia The mean refraction was + 3 0 (range + 1 0 to + 7 0)

We have included only those patients in whom the penalization therapy is considered completed or who have received it for at least one year (Completed therapy is taken to mean that the amblyopia has been cured or that the treatment has proved ineffective or that it has been given up by the patient and parents)

Therapeutic Methods

The following three penalization methods were employed

(a) *penalization for near*

fixing eye treated with atropine 1% daily and normal correction amblyopic eye with over correction (as a rule by 2 dioptres)

(b) *penalization for distance*

fixing eye treated with atropine 1% daily and over correction (as a rule by 3 dioptres) amblyopic eye with normal correction

(c) *total penalization*

fixing eye treated with atropine 1% daily and under correction (as a rule by 5 dioptres) amblyopic eye with normal correction

In a few cases with accommodative convergence excess the penalization for near was modified to so called *selective penalization* meaning only that the amblyopic eye was fitted with a bifocal glass In the maintenance therapy the penalization for distance

was modified in a few cases to *alternating distance penalization* meaning that the patients were provided with two pairs of spectacles one pair over correcting the left eye and the other pair the right eye by 3 dioptres The spectacles are to be worn alternately every other day and thus counteract a recurrence of the amblyopia (This form of distance penalization of course is practised without instillation of atropine)

All but two of the patients were treated primarily by penalization for near In three cases this was followed by penalization for distance viz when the visual acuity did not get better than 6/12–6/18 on penalization for near In several cases (six) total penalization was also used in an attempt to cope with residual amblyopia or as the primary method of penalization in very deep amblyopia (two patients with an initial visual acuity of less than 6/60)

The mean duration of the treatment was 10 months range 5–20 months

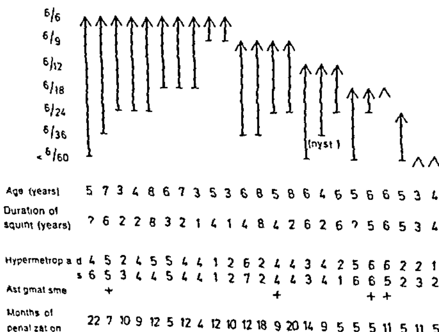


Fig 1

Individual visual gains marked by arrows The base of the arrow indicates the initial value i e the amblyopia after an average of 8 months ineffective occlusion therapy per ... al in 20 cases constant in three) The tip of the arrow indicates the visual acuity after an average of 10 months penalization therapy The patients ages and the duration of squinting at the time of initiating penalization are stated Astigmatism is listed only if it is more than 1 dioptre in the amblyopic eye

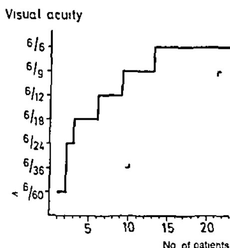


Fig 2
Step diagram representing the total amblyopia (mean 6/24) after the unsuccessful occlusion therapy and prior to the penalization therapy (broken line) as well as the total therapeutic result (solid line – mean 6/9)

Therapeutic Results

Fig 1 presents the individual results It is apparent that in 17 out of the 23 patients quite a considerable visual improvement was obtained although this material is at the heavy end of an amblyopia clientele The mean visual acuity in the amblyopic eye increased from 6/24 to more than 6/9 It is also apparent from Fig 1 that in two patients who had a visual acuity of less than 6/60 at the institution of penalization the amblyopia proved refractory to all forms of penalization presumably because of a real eccentric fixation or organic amblyopia (As already mentioned considerable improvement was obtained in 17 of the 23 patients Out of the total material three patients gave up before maximal improvement could be expected and in another three cases the penalization was abandoned as ineffective by the Clinic at the end of 5–11 months) Among the successfully treated 17 patients five are still being treated (now in their 2nd year) because further visual improvement is still possible

Fig 2 gives the total results in a diagram It must be emphasized that eight of the patients who obtained 6/6 in the previously amblyopic eye alternate spontaneously This high incidence of spontaneous alternation in this heavy material of amblyopia at the completion of treatment may be due to the patients obtaining in the course of the penalization therapy the so called spatial balance i e they use one eye for near and the other eye for distance according

to which form of penalization is used Thus the patients get accustomed to incessantly changing between the right and left eye

Follow up studies have shown that on an average half the visual improvement obtained by conventional occlusion therapy will be lost in the long run (Gregersen & Rindziunski 1965 Fletscher & Zimmermann 1968)

It is possible that spontaneous alternation may be easier to obtain by penalization than by conventional occlusion and that accordingly the long term results will perhaps prove a little better after penalization therapy than after the conventional occlusion

Discussion

As already mentioned conventional occlusion therapy has proved by far the best method of treating squint amblyopia in our Clinic Therefore we have used the penalization methods only when occlusion therapy has been given up by the patients and parents In a few cases however the penalization methods are preferable to conventional occlusion therapy viz in patients with recurrent occlusion amblyopia and in patients with latent nystagmus which becomes manifest on occlusion

In our small material we have not found the penalization methods better than correct long lasting and permanent conventional occlusion i e when this treatment is accepted by the patients and parents It should be mentioned that we define a correct continuous occlusion therapy as whole day occlusion therapy by adhesive tape carried through continuously for at least as many months as the years the child has lived with a maximum of one weekly day off

The penalization usually proved easy to carry through (only three patients and parents giving up) The children spontaneously keep their spectacles on as the spectacles improve their visual situation and thereby *act as a positive measure It must* be emphasized also that when the children take their spectacles off they often suffer diplopia because the penalization inhibits suppression and tends to maintain a possibly normal retinal correspondence

It is a *sine qua non* for attaining a therapeutic success by penalization for *near that* the fixing atropinized eye is inferior to the favoured amblyopic eye *in* near vision It is important therefore to *check at all* times that near vision in the eye with atropine 1% once daily and *with distance correction is poorer than* the near vision in the amblyopic eye with 2 dioptres over correction Accordingly the cycloplegia of the fixing dominant *eye has to* be checked at regular intervals In some few patients with moderate to marked hypermetropia we have observed that the accommodation of the fixing eye was far from being

abolished in spite of atropine 1 % once daily for several months even though the pupil was maximally dilated and inactive to light In such cases atropine drops 1 % several times daily in the fixing eye and 3 dioptres over correction of the amblyopic eye may be tried (As regards the effect of penalization on retinal correspondence and squint angle see Gregersen et al 1974)

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FOVEAL SPECTRAL THRESHOLD IN TOBACCO AMBLYOPIA

BY

S K. BHARGAVA and C I PHILLIPS

The foveal thresholds for red green and blue colours of twelve patients with tobacco amblyopia and twelve age matched normal controls were measured with the Tubinger perimeter The difference in foveal thresholds between the patients and controls was highly significant for red (t 17 5 $P < 0\ 001$) just significant at 5% level for green (t = ?.31) and was not significant for blue (t = 2 07 $P > 0\ 05$) The difference in thresholds between patients and controls for red was significantly greater than that for green and blue The difference in threshold between patients and controls for green did not differ significantly from that for blue Thus in tobacco amblyopia the foveal sensitivity to red was far more reduced than for the green or blue

Key word foveal threshold – tobacco amblyopia – Tubinger perimeter

Acquired dyschromatopsia of red green type is one of the diagnostic features of tobacco amblyopia (Carroll 1935 Herbolsheimer 1942 François & Verriest 1961 Anley 19 0 Chisholm et al 19 0) Depressed sensitivity to red in the centro caecal area of the field of vision can be demonstrated long before it is apparent with a white target (Hutchinson 1887 Nettleship 188, Traquair 1930

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Carroll 1935 Heaton McCormick & Freeman 1958 Silvette Haag & Larson 1960) The colour perimetry is complex and the field defect found with a red target in tobacco amblyopia is not only due to the colour defect present but also the decreased luminosity of the coloured target (Harrington 1964) and also possibly to the decreased retinal sensitivity in tobacco amblyopia

Foveal threshold measurement is one of the methods for evaluation of the sensorial degradation in ocular disease (Zanen 1972) and is a very sensitive test of macular dysfunction

This communication deals with the investigation of the achromatic threshold of coloured targets in tobacco amblyopia

Material and Methods

Twelve male patients (mean age 60 7 years) with diagnosed tobacco amblyopia and 12 normal controls matched with sex and age (mean age 60 9 years) participated in this study The patients were all pipe smokers with acquired bilateral defect of visual acuity associated with bilateral centrocaecal scotoma and acquired dyschromatopsia of Red Green type Foveal threshold for coloured targets were measured with a Tubinger perimeter

The Tubinger perimeter is a hemispherical bowl perimeter with the surface of the bowl 33 cm away from the patient s eye Foveal thresholds were measured by using a test target of 42 min of arc in size The test target projector is incorporated with red green and blue coloured glass filters (filter by Schott RG2 GG14 and BG12) for colour stimulus The maximum light intensity for the white test target is 1000 asb which can be reduced in 80 equidistant logrithmic stages to 0 00001 asb by means of two dials one containing a series of seven and the other a series of nine grey filters The stages of light intensity are read by position code numbers 00 to 79 corresponding to 1000 asb to 0 00001 asb respectively

A constant white background illumination of 10 asb was used throughout the test It was performed binocularly in a completely dark room and the subject was allowed to adapt to this level of background illumination for 5 min before commencing the test A rest of 5 min was also allowed in between the different colour threshold measurements i e red green and blue and this was also the order in which these colours were presented

The subject s fixation was maintained in the form of four small points uniformly distributed around an external diameter of two degrees the individual

points being 10 min of arc in diameter and the light intensity of the fixation spots was 10 asb

The test target at a luminance considerably less than that of the threshold value was presented at the centre of the fixation spots and the luminance of the test target was gradually increased until the subject just became aware of it The position on the dial gave the threshold value in a logrithmic scale

Results

The foveal thresholds of the patients as well as those of the normal controls as measured with the Tubinger perimeter are presented in Table I

Table I

Foveal threshold values of 1? patients and 1? normal controls with Tubinger perimeter

Patient no	Age		Threshold red		Threshold-green		Threshold-blue	
	Patient	Control	Patient	Control	Patient	Control	Patient	Control
1	78	78	14	?0	11	10	6	7
	58	58	15	2?	10	1?	10	14
?	74	74	7	18	13	13	7	6
4	64	?1	9	?0	7	13	6	11
	6?	66	13	?0	1?	14	8	10
6	4	48	15	23	14	18	4	1?
	61	61	14	[illegible]	10	16	4	16
?	64	6?	14	??	14	14	11	10
9	54	54	13	[illegible]	13	14	10	10
10	[illegible]	68	15	[illegible]	11	1?	9	10
11	?9	0	14	[illegible]	13	16	8	10
1?	?8	?	16	1	16	13	16	13
				??		14		12

Table II

Analysis of variance for significance between the differences in thresholds of the patients and the normal controls for different colours

Source	Sum of squares	d f	Mean square
Patient control pairs	956 67 – 729 0 = 227 65	11	20 697
Colours	999 50 – 729 0 = 270 50	2	135 25
Interaction	1426 – 956 67 – 999 5 + 729 = 198 83	22	9 038
Total	1426 – 729 = 697	35	

The paired *comparison t test was used to see if the patients* thresholds differed significantly from those of the normal controls for red green and blue colours The results show that the difference between the patients and the controls is highly significant for red (t = 17 35 d f 11 $P < 0\ 001$) just significant for green (t = 2 31 d f 11 $0\ 05 > P > 0\ 02$) and not significant for blue colour (t = 2 07 d f 11 $0\ 1 > P > 0\ 05$)

The analysis of variance test was used to find out if there was a significant difference between the differences in thresholds of the patients and the normal controls for the different colours (Table II)

The test shows that the differences in threshold values for red green and blue are significantly different (I ratio = 14 96 with 2 and 22 d f $P < 0\ 01$) Further the studentized range test (Wetherill 1967) was used to see which means for the differences between patients and controls for the three colours differ significantly from each other i e whether the differences between red and green red and blue or green and blue colours are significantly different A range is calculated such that this range will only be exceeded by any differences in the means for the three colours with a probability of 5 % if there is no difference between the colours The range is calculated as follows

$$\text{range} = q\,\alpha\ \ k\,\nu \sqrt{\frac{V_1}{J}}$$

where $q\,\alpha\ k\,\nu$ = value from tables of the studentized range corresponding to the probability of α where k samples are considered and ν is the degree of freedom of the interaction sum of squares

V_1 = mean square for interaction

J = number of results in the mean for the colours

In this case $\alpha = 0\,0$ $k = 3$ $\nu = 9?$ $V_1 = 9\,038$ from the analysis of variance table and J 1? since there are 1? patient control pairs for each colour From tables of the studentized range (Documenta Geigy 1970) q 0 0ↄ 3 ?? = 3.5ↄ

$$\text{Therefore range} = q\ 0\,0ↄ\ 3\ ??\ \sqrt{\frac{V_1}{J}} = 3\ ↄ\ \sqrt{\frac{9\,038}{1?}} = 3\,08$$

Any difference in the means for colours greater than 0 38 is significant at the 5 % level Hence the differences in the means between red and green and between red and blue are significant The difference in the means between green and blue is not significant

Discussion

In another study (Bhargava 1973) it was shown that the type of colour defect in tobacco amblyopia is that of extreme protanomaly as diagnosed by the Pickford Nicolson anomaloscope and that the red colour mechanism may be at fault The comparison of patients thresholds for red green and blue colours with those of normal controls in this study further supports this view The foveal sensitivity to red colour was far more diminished than to the green colour

Marré & Marré (1972) found that in retinal diseases the sensitivity of the fovea decreases as the wavelength increases whereas in optic nerve diseases the reduction in sensitivity is constant over the whole spectrum Hong (1957) also describes the shift of the maximum of luminosity curve towards the shorter wavelength in the retinal group of diseases and the luminosity curve remains normal in the neural group of diseases Our findings are consistent with the former phenomenon in that the foveal sensitivity decreases significantly for red (longer wavelength) as compared with green or blue (shorter wavelength) and it is reasonable to suppose that in tobacco amblyopia the lesion could be at retinal level Also Junemann & Damaske (1968) have shown that an episode of smoking by habitual non smokers has some effect on the retina in that it causes a reduction in the amplitude of the b wave in the ERG

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RETINAL HAEMORRHAGES IN THE NEWBORN

Comparison of delivery by forceps and by vacuum extractor

BY

NIELS EHLERS IB KRARUP JENSEN and KARL BROGÅRD HANSEN

Retinal haemorrhages were studied in a consecutive series of 99 babies del vered by forceps 94 delivered by vacuum extractor and 907 delivered spontaneously

The incidence of haemorrhages for spontaneously delivered was 27.5 % for those delivered by forceps 35% and for those delivered by vacuum *extractor 64%* The incidence of haemorrhages in the group of vacuum extractions was sign ficantly higher than among either the group of forceps extractions or the spontaneous del veries In addition more severe haem rrhages were found in the group of vacuum extractions Influence on the incidence of haemorrhages by factors other than type of delivery could not be verif ed statistically

Key words retina – retinal haemorrhages – newborns – artificial delivery

Retinal haemorrhages which occur fairly frequently in the newborn have been the object of several studies since the first descriptions of a century ago In the Department of Obstetrics Fødselsanstalten Århus a comparative study of deliveries by means of forceps or vacuum extractor was planned and an assessment of the incidence of retinal haemorrhages was a natural part of

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this. A consecutive series of deliveries was studied. Extraction by forceps was performed on uneven dates, vacuum extraction on even dates. A full description of this study is given elsewhere (Brogård et al., to be published). Below a report is given on the ophthalmological findings and how they are influenced by some obstetrical factors.

Material

The ophthalmological study comprised 413 newborns, 221 males and 192 females. Ninety-nine babies were delivered by forceps, 94 by vacuum extractor, while in 13 cases a failed vacuum extraction was followed by forceps delivery. In order to have a group of normal deliveries for comparison the first baby weighing more than 2500 g to be delivered spontaneously after each artificial delivery was examined. This group comprised 207 children. The comparability of the groups delivered by forceps and by vacuum extractor is demonstrated by Table I.

Table I
Characteristics of consecutive, random selected series of delivery by forceps or by vacuum extractor

	99 deliveries by forceps	94 deliveries by vacuum extractor
Girls/boys	31/68	44/50
First born	87 %	86 %
Duration of labour > 12 h	42 %	39 %
Second stage labour > 1 min	70 %	68 %
Pathologic position of foetus	23 %	23 %
Reduced Apgar score	29 %	16 %
Maternal disease		
before pregnancy	9 %	6 %
during pregnancy	21 %	18 %

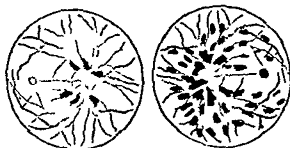


Fig 1

Examples of retinal haemorrhages In the right eye few scattered haemorrhages in the left numerous retinal haemorrhages and a macular haemorrhage

Ocular examination Tropicamide (0 5 %) and metaoxedrine (10 %) were instilled twice with an interval of 15 min Direct ophthalmoscopy through a Richardson lens was performed 1 2 hours later within 30 hours after delivery The findings were recorded in a diagram (Fig 1) and grouped as (1) No haemorrhages (2) Few scattered retinal haemorrhages (3) Numerous retinal haemorrhages (4) Macular haemorrhages or (5) Prepapillar haemorrhages When haemorrhages were found the examination was repeated on the seventh day Haemorrhages present at the second examination were followed until they had disappeared

Results

Incidence of retinal haemorrhages Of the 413 children examined 165 had retinal haemorrhages (39 9 %) Thirty eight of the 99 children delivered by forceps (38 %) showed haemorrhages while 60 of the 94 children delivered by vacuum extraction (64 %) showed haemorrhages Nine of 13 children in whom a failed vacuum extraction was followed by forceps delivery had retinal haemorrhages In the group of control children 5 of 207 (27.5 %) revealed this pathology Statistical calculations by means of the χ test showed that significantly more haemorrhages occurred among the vacuum extractions ($P < 0.0005$) than among either controls or forceps extractions The difference between newborns delivered by forceps extractions and spontaneously delivered was not statistically significant ($0.05 < P < 0.1$)

Appearance localization and fate of retinal haemorrhages Usually a limited number of flame shaped or rounded intraretinal haemorrhages occurred localized peripapillarily or scattered over the posterior part of the fundus (Fig 1) Occasionally the number of haemorrhages was very large the entire posterior fundus being covered The vessels always looked normal an exudates were never seen Special localizations were macular and prepapillar In a few cases peripheral choroidal haemorrhages were noted

The findings in the studied series grouped according to the classification mentioned are shown in Table II which also includes the findings at the examination on the seventh day While the scattered retinal haemorrhages have mainly disappeared after 6 days the macular and prepapillar haemorrhages persist These haemorrhages were followed up and they all disappeared within a few months leaving no permanent visible damage One of the prepapillar haemorrhages in a child delivered by vacuum extraction was of the type described by Brendstrup (1969) localized to the primary vitreous

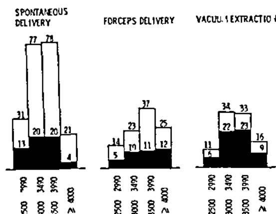
Table III shows how the time of examination after delivery influences the frequency of haemorrhages No statistically significant differences were found between the frequency of haemorrhages observed in less than 12 hours 12 to 24 hours and 24 30 hours after delivery

Influence of birth weight and sex Fig 2 shows the material grouped according to birth weight In none of the three groups was there any statistically significant correlation between birth weight and frequency of haemorrhages In

Table II
Localization of retinal haemorrhages

	Spontaneous delivery		Delivery by forceps		Vacuum extraction	
	1st day	7th day	1st day	7th day	1st day	7th day
Few scattered non macular	44	6	30	4	32	7
Uniformly distributed	4	2	1	1	-	-
Macular	7	7	-	6	15	16
Prepapillar	3	2	1	1	4	4

The four groups of haemorrhages are not mutually exclusive



Fig

Retinal haemorrhages and birth weight White columns total number black columns number with retinal haemorrhages Birth weight in grams In none of the three group was there any statistically significant correlation between birth weight and frequency of haemorrhages (χ tests)

Fig 3 the series are divided according to sex In each sex more haemorrhages occurred after vacuum extraction ($P < 0.001$) There were no differences between the frequency of haemorrhages in female and males

Parameters related to labour In 69 of the 99 cases delivered by forceps more than one pull was needed Among these 69 cases haemorrhages were found in

Table III

Influence of period of time between delivery and examination

Time of observation (hours)	Spontaneous delivery			Delivery by forceps			Vacuum extraction		
	Total no examined	No of haem		Total no examined	No of haem	•	Total no examined	No of haem	•
< 1	56	14	[illegible]	28	8	29	17	10	59
1 24	119	30		56	[illegible]	45	63	40	64
> 24	33	13	39	15	5	33	14	10	[illegible]
Total	208	3	.5	99	38	38	94	60	64

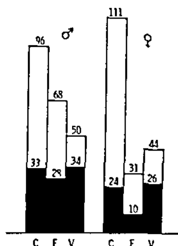


Fig 3

Retinal haemorrhages and sex White columns total number black columns number with retinal haemorrhages C control = spontaneous delivery F forceps delivery V vacuum extraction *In both sexes more haemorrhages occurred after vacuum extraction* ($P < 0.001$) No differences between frequency of haemorrhages in females and males

26 (38 %) In 84 of the 94 vacuum extractions the time of pull was longer than 1 min Among these 84 cases haemorrhages were found in 58 cases (63 %) As already mentioned 9 of the 13 cases in which a failed vacuum extraction was followed by forceps showed retinal haemorrhages (69 %)

The Apgar score of the newborn was noted in the forceps and the vacuum groups Among the forceps delivered where 38 % had retinal haemorrhages 29 had an Apgar score under 10 Of these 29 children 15 (52 %) had retinal haemorrhages This frequency does not differ statistically significantly from that of the entire forceps group ($0.05 < P < 0.1$) Among the 94 vacuum extractions 15 had an Apgar score under 10 Nine of these had retinal haemorrhages (60 %) a frequency corresponding to that for the entire group of vacuum extractions (60 of 94 64 %)

The duration of labour the position and the presentation of the foetus had no relation to the incidence of haemorrhages

Maternal diseases age of mother previous deliveries The material has been analysed with respect to major diseases before pregnancy and to major complications during the pregnancy The number of cases was small but neither condition appeared to influence the incidence of haemorrhages in any of the groups mentioned Fig 4 shows the material grouped according to the age of

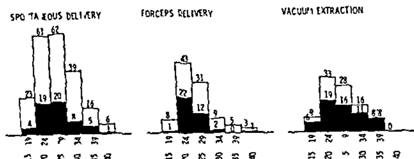


Fig 4

Retinal haemorrhages and age of mother White columns total number black columns number with retinal haemorrhages In none of the three groups could any heterogeneity be demonstrated by the χ test

the mother In none of the three groups could any heterogeneity be demonstrated by the χ test The material contained 259 first born babies of which 110 showed haemorrhages (42 4 %) Among 141 subsequent babies 47 showed haemorrhages (33 4 %) The difference between first and subsequent babies is not statistically significant ($0\,05 < P < 0\,1$) Fig 5 shows the material divided into first born and subsequent babies

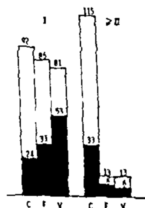


Fig 5

Retinal haemorrhages and parity White columns total number black columns number with retinal haemorrhages C control = spontaneous delivery F forceps delivery V vacuum extraction No differences between first and subsequent babies could be demonstrated

Discussion

The problem of retinal haemorrhages in the newborn has been the subject of numerous investigations during the last century The earlier literature has been excellently reviewed by Sanchez Ibanez et al (1963) Recent studies have been published by Krauer Mayer (1965) Krebs & Jäger (1966) Schenker & Gombos (1966) Neuweiler & Onwudiwe (1967) Sezen (1970) Weiden (1970) Baum & Bulpitt (1970) Planten & Schaaf (1971) and Pommer (1972) In the Scandinavian literature the subject has been studied by Bjerrum (1884) Wille (1944) and Brendstrup (1969)

Various authors have reported overall incidences of retinal haemorrhages to vary between a few and more than 40 % Recent larger series agree upon an incidence of about 30 % The large variation may be partly due to different observation times as all authors agree that most of the haemorrhages disappear within a few days (Table II) In agreement with Weidens results no correlation could be demonstrated in our series between incidence of haemorrhages and time of observation within the first 30 hours

Classifications of haemorrhages according to morphology have been numerous However the clinical significance seems to have more connection with the rate of disappearance probably corresponding to a localization in or in front of the retina

General agreement exists on there being a higher incidence of haemorrhages after delivery by vacuum extraction (Sanchez Ibanez 1963 Krauer Mayer 1965 Krebs & Jäger 1966 Schenker & Gombos 1966 Neuweiler & Onwudiwe 1969 Weiden 1970) about 60 % After delivery by forceps the incidence is generally reported to be slightly higher than after spontaneous delivery although in some series no difference is found The figures of the present series correspond to those of the literature after spontaneous delivery 27 5 % haemorrhages after forceps 38 % after vacuum extraction 64 % It is emphasized that the present series of deliveries by forceps or by vacuum extractor is the only one in which the two groups are directly comparable (consecutive randomly selected) Statistical significance can be demonstrated for the difference between delivery by vacuum extraction versus forceps or spontaneous delivery but not for the difference between delivery by forceps and spontaneous delivery Furthermore a relatively large part of the haemorrhages seen after delivery by vacuum extraction was of the preretinal type a tendency also noted by others If late sequelae can be expected it should be after this type of haemorrhage

Several authors have reported a correlation between incidence of haemorrhages and various obstetrical factors such as birth weight and sex duration

and severity of labour complications of delivery Apgar score of the newborn and first and subsequent births In this series no such correlations have been statistically verified which is in close agreement with the results of Weiden (1970)

Numerous attempts have been made to explain the pathogenesis of retinal haemorrhages in the newborn A generalized haemorrhagic tendency has been supposed but seems to be of little or no significance (Planten & Schaaf 1971) Several authors have stressed the importance of haemostatic or dynamic factors The incidence of retinal haemorrhages is low after Caesarean section r breech delivery This suggests that pressure on the head during delivery or compression of the thorax when the head has been delivered may be of importance Retinal stasis by thorax compressions seems to be the most direct explanation the mechanism resembling that of retinal haemorrhages after thorax compressions in adults and after pressure reducing ocular surgery In the literature no single theory has been adopted

The prognostic significance of retinal haemorrhages remains an open question From the literature there appears to be no association between retinal haemorrhages and brain damage but it is still possible that sub clinical alterations in the central nervous system occur in children with retinal haemorrhages The possible role of macular haemorrhages in the development of amblyopia has been studied in only a few series It is intended to follow up the children in the present series who had macular haemorrhages

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ON THE TREATMENT WTH PROFLAVINE OF HERPES SIMPLEX OR VACCINIA INFECTED GMK CELLS

An Electron Microscopic Study

BY

TÕNIS LUNT SVEN ERIK G NILSSON AND STIG JEANSSON

Green monkey kidney (GMK) cells were inoculated with vaccinia or herpes simplex virus and the effect of photodynamic treatment with proflavine and light was studied in the electron microscope Whereas virions were abundant in all untreated cultures (incubated with virus for 15 hours or m re) no virus particles could be found in the treated ones Proflavine and light in the amounts used seemed to exert a certain degree of t xicity on the GMK cell cultures but virus reproduction was *effectively* stopped by the treatment Preliminary results on photodynamic treatment of human herpes keratitis are mentioned

K y word herpes simplex – vaccinia – proflavine – electron microscopy

Herpes keratitis is a severe and rather common eye disease Despite the recent advancements in therapy (idoxuridine cryotherapy etc) there are many cases n whi h satisfactory healing cannot be achieved Thus it is of great interest that new ways are being tried experimentally and clinically

It is well kn wn that virus can be photodynamically inactivated in the pre ence of certain dyes (Hiatt et al 1960) Wallis & Melnick (1964) showed that

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Green monkey kidney (GMK) cells were inoculated with *vaccinia or herpes* simplex virus and the effect of photodynamic treatment with proflavine and light was studied in the electron microscope. Whereas virions were abundant in all untreated cultures (incubated with virus for 15 hours or more) no virus particles could be found in the treated ones. Proflavine and light in the amounts used seemed to exert a *certain degree of* toxicity on the GMK cell cultures but virus reproduction was effectively stopped by the treatment. Preliminary results on photodynamic treatment of human herpes keratitis are mentioned.

Key words herpes simplex – vaccinia – proflavine – electron microscopy

Herpes keratitis is a severe and rather common eye disease. Despite the recent advancements in therapy (idoxuridine cryotherapy etc.) there are many cases in which satisfactory *healing* cannot be achieved. Thus it is of great interest that new ways are being tried experimentally and clinically.

It is well known that virus can be photodynamically *inactivated* in the presence of certain dyes (Hiatt et al. 1960). Wallis & Melnick (1964) showed that

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Fig. 1
Normal CMK cell with part of the nucleus (N) and some mitochondria (M) × 4 000

certain viruses were inactivated *in vitro* after exposure to light in the presence of either toluidine blue proflavine or neutral red Experimental herpes keratitis in rabbits was successfully treated with proflavine and light by Moore et al (1972) whereas Varnell & Kaufman (1973) found the effect of this treatment minimal though definite However neutral red and light seemed to have no effect on the natural course of experimental herpes keratitis in rabbits (Thomas et al 1973) No clinical studies on the effect of proflavine on human herpes keratitis have been published

The present paper reports the results of electron microscopic investigations of the effect of proflavine and light on cell cultures infected with herpes virus or with vaccinia virus Preliminary results of photodynamic treatment of human herpes keratitis will be briefly mentioned in the discussion

Material and Methods

Green monkey kidney cells (GMK – AH 1) (Gunalp 1965) were inoculated with herpes simplex virus type 1 or with vaccinia virus The concentration of virus was chosen so as to produce a definite cytopathogenic effect visible within 24 hours The viruses were allowed to adsorb for 3 hours After that the medium was changed twice Some of the cell cultures were prepared for electron microscopy 12 15 18 21 or 24 hours after inoculation and then investigated with respect to the presence of virus particles and cellular damage Other cell cultures were treated with proflavine beginning at different intervals (3 6 or 9 hours) after inoculation (The treatment of two samples of vaccinia infected cells was not begun until 12 hours after inoculation) These cultures were incubated for 3 hours in a medium containing proflavine hemisulphate (SIGMA P 2508 further analyzed in Sweden as to purity) diluted 1 300 000 and were kept in reduced daylight After rinsing the cell cultures three times with a medium free of proflavine the cultures were exposed to light from two daylight fluorescent tubes (Atlas 40W/33 cool white made in Great Britain) at a distance of 5 cm for 5 min Treated cell cultures were prepared for electron microscopy at 24 hours after inoculation with virus i e at 12 15 18 or 21 hours after the beginning of the treatment

Also non infected cell cultures were examined Some of them were incubated with proflavine at the same time intervals as the infected cell cultures and were also exposed to light from the fluorescent tubes Other cultures were incubated with proflavine but exposed only to reduced daylight Some uninfected cell cultures were exposed to light from the fluorescent tubes without pretreatment



F g 1
N mal CMK ell with part f the nu l u (N) and some m tochondria (M) × 4 000

with proflavine These cell cultures were then studied ultrastructurally as to possible damaging effects of the dye and/or the exposure to light

The material was fixed in 4 % glutaraldehyde postfixed in 1 % osmium tetroxide dehydrated in acetone and embedded in Vestopal W Ultrathin sections were examined in a Philips 300 electron microscope

Observations

The cytoplasm of normal GMK cells was found to contain mitochondria rough surfaced endoplasmic reticulum and a great many clusters of free ribosomes (Fig 1) The Golgi apparatus was well developed Tubular and stranded material was also present Electron dense bodies of about the same size as the mitochondria and often containing myelin figures (as B in Fig 2) could be observed The nuclei generally were round or ovoid and contained rather evenly dispersed chromatin except for the most peripheral parts where a narrow zone with more chromatin could be seen close to the nuclear membrane Nucleoli were seen in many nuclei Normal cells exposed to light from fluorescent tubes did not differ from the description given above

Cells incubated with proflavine whether exposed to light from the fluorescent tubes or not differed from untreated cells in one respect the number of cells containing electron dense bodies with myelin figures seemed higher and the number of such bodies per cell also seemed higher (Fig 2) Furthermore it can be noted that in preparations of cell cultures treated with proflavine and light long before fixation (e g 18–21 hours before fixation) a certain amount of cell fragments could also be found Such cell fragments were seldom seen in untreated preparations or in preparations treated with proflavine but without exposure to extra light

Cell cultures infected with vaccina virus were seen to contain intracytoplasmic particles structurally well corresponding to vaccina virions (Fig 3) Different stages of the virus maturation process could easily be demonstrated in cultures that had been infected for 18 hours or more (Fig 3) Also several electron dense bodies with myelin figures were found In the case of cultures infected for 15 or 12 hours the demonstration of vaccinia virions required more extensive search and virions were found mainly in damaged cells or in cell fragments

Herpes simplex infected cell cultures showed vacuolization of the cytoplasm and cell fragmentation in those samples that were inoculated 24 or 21 hours before fixation Electron dense bodies were abundant Many mitochondria



Fig

CMK ll c l w th p ll n an l l ght w th out p us rus inoculat n F xed f t n m p f hours aft r th b g nning f th treatment El ctron dense t l es B r n m n the m t ch ndria (M N nu leus 30,000)



Fig 3
GMK cell inoculated with vaccinia virus 24 hours prior to fixation V virus particle M mitochondrion × 51 000

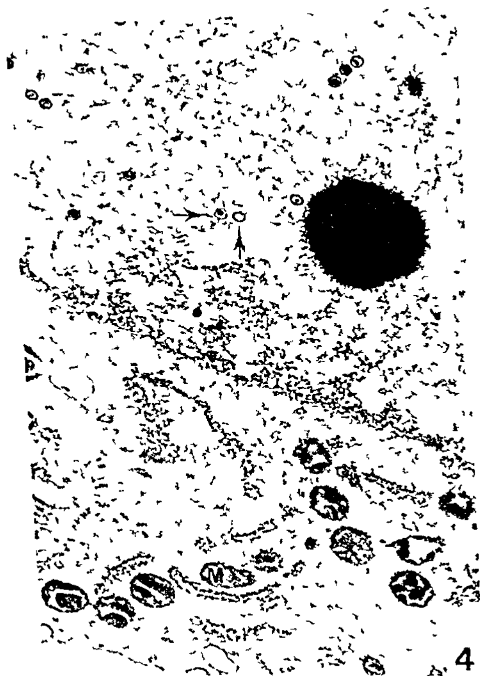


Fig. 4

CMK cell inoculated with herpes simplex virus 4 hours prior to fixation. Arrows: virus particles in the nucleus. M: mitochondria with wide internal spaces. × 41,000

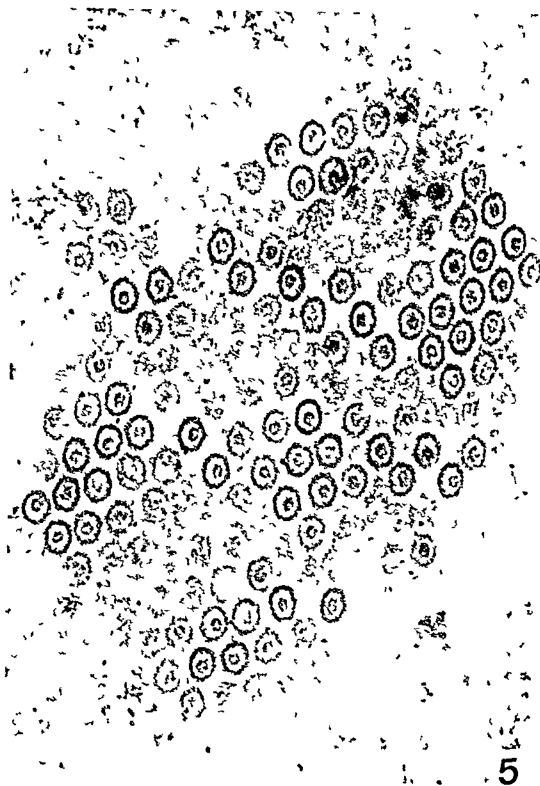


Fig 5
GMK cell inoculated with herpes simplex virus 24 hours prior to fixation showing an intranuclear virus crystal × 85 000



Fig. 6

CMK cell inoculated with vaccinia virus 4 hours prior to fixation and treated with proflavine and light 12 hours after inoculation. No virus particles are seen. Electron dense bodies (B) are found among the mitochondria (M). N nucleus × 33 000

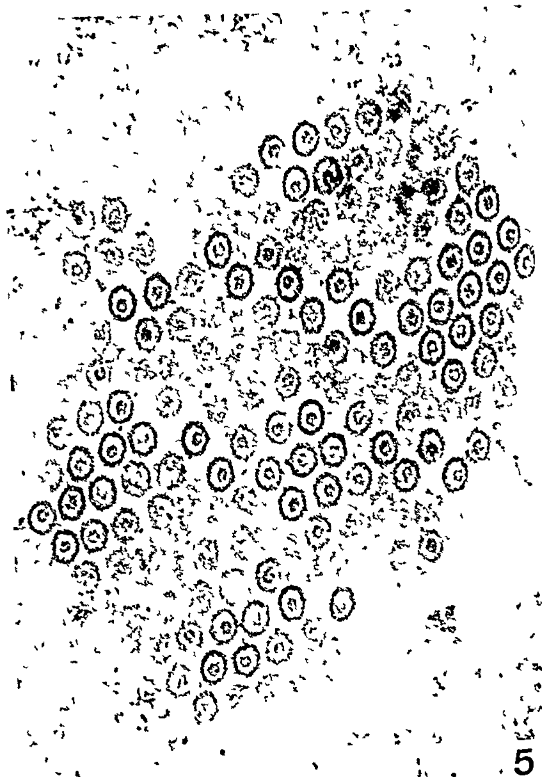


Fig. 5
GMK cell inoculated with herpes simplex virus 24 hours prior to fixation showing an intranuclear virus crystal × 85 000



Fig 7

CMK cell inoculated with herpes simplex virus 24 hours prior to fixation and treated with proflavin and light 9 hours after inoculation No virus particles are seen The mitochondria (M) appear changed B electron dense body N nucleus × 42 000

showed wide internal spaces (Fig 4) The nuclear chromatin seemed more sparse than in normal cells In the nuclei particles structurally well corresponding to herpes simplex virions were found (Figs 4 and 5) These structures could sometimes be seen in crystal arrangement in the nuclei (Fig 5) They were also found in the cytoplasm and extracellularly where they were enclosed in a membrane envelope Cells that had been infected for 18 or 15 hours did not show any virus like particles in the cytoplasm or extracellularly but in the nuclei such particles were easily found Cell cultures infected 12 hours before fixation however did not obviously differ from uninfected ones and no virus like particles could be found in the cells

Cells infected with herpes simplex virus or vaccinia virus and treated with proflavine and light at different intervals (3 9 or 12 hours) after virus inoculation did not obviously differ from uninfected cells treated with proflavine and light Thus electron dense bodies were frequently seen (Figs 6 and 7) It should be noted that mitochondrial changes in the form of wide internal spaces occurred in cells inoculated with herpes virus 9 hours before treatment (Fig 7) No virus like particles could be found whether the treatment with proflavine was begun early or late after virus inoculation (Figs 6 and 7)

Discussion

Although photodynamic inactivation of viruses *in vitro* (Hiatt et al 1960 Wallis & Melnick 1964) and in rabbit herpes keratitis (Moore et al 1972 Varnell & Kaufman 1973) was described earlier no ultrastructural studies have been published on the subject It ought to be of interest to know what happens to the virus particles as well as to the cellular structures The structure of vaccinia virus (Morgan et al 1954) and herpes simplex virus (Wildy et al 1960) is well known

In the present investigation it was found that following treatment with proflavine and light virus particles could no longer be demonstrated in the CMK cells Whether the viral material had disappeared from the cells or was changed so that it could no longer be identified ultrastructurally is not known The increased number of electron dense bodies seen in cells treated with proflavine and light whether infected or not must be interpreted as a sign of a slight degree of cell toxicity of the substance The toxicity seemed to depend at least in part on light sensitization of the cells since the cell damage in the form of fragmentation appeared more pronounced if the cultures were not only exposed to reduced daylight but also received extra light from fluorescent tubes

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Since the virus reproduction obviously was stopped by the treatment since the toxic effects seemed to be only minor and since in one study good results were achieved on rabbit herpes keratitis (More et al 1972) we have recently tried proflavine and light also on nine cases of human herpes (dendritic) keratitis (Proflavine which formerly was used against bacterial infections is registered as eye drops in Martindale *The Extra Pharmacopoeia* 26th ed) All nine corneas healed although two of them very slowly It is well known however, that herpes keratitis can show spontaneous healing and therefore since our material is still very small it is too early to draw any conclusions as to the therapeutical value of proflavine and light on human herpes keratitis The procedure of treatment and the results will be presented in detail when the number of patients is considered sufficient Chronic herpes virus infection of the lacrimal gland and conjunctiva of man (Kaufman et al 1968) can hardly be affected by photodynamic treatment and may well give rise to recurrences Further studies on human herpes keratitis will show the effectiveness of proflavine as compared to that of idoxuridine chemical cauterization cryotherapy etc

Acknowledgments

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A scleral plaque is a well defined spot on the sclera due to scleral transparency causing the underlying uveal tissue to shine through with a greyish tinge This spot is best studied in the slit lamp

In 19,3 I introduced the concept of *preplaques* which I believed to be a precursor of scleral plaques

A preplaque is best *recognized without magnification* by light from an ordinary pencil flashlight It manifests itself as a greyish band anterior to the insertion of one of the horizontal eye muscles The phenomenon is not detectable by direct illumination through the slit lamp

In some eyes with a prelaque we may find in addition a true scleral plaque within the preplaque area

The present paper deals with the proper scleral *plaque*

Different names have been given in the literature to the scleral plaque

Senile hyaline degeneration of the sclera (Pur 19ɔɔ)
Hyaline senile scleral plaques (Roper Vail Boshoff 1945)
Senile Scleralverdunnung (Kiss 1934)
Umschreibener Lederhautschwund (Kyrieleis 1939)
Senile Entartung der Lederhaut (Pillat Gasteiger 1933)
Developmental bilateral mesial superficial intagliated deficiency of the sclera (Graves 1941)
Focal senile transluency of the sclera (Cogan 1956 Hogan 19,1)

Historical review

Rolandi was the first to describe scleral plaques He published three cases in 191ɔ The first aged 7? had a spot nasally in one eye the second aged ,, a spot nasally in both eyes and the third aged 6 5 × 2 mm large spots in front of all four horizontal muscles (quoted by Roper)

On June 19 1933 Pillat reported a case of scleral plaques in a paper read before the Ophthalmological Society in Vienna He claimed to be the first to have described scleral plaques doubting whether Rolandi s case could be classified as such I illat however had not read Rolandi s original paper

About ɔ0 clinical cases have been published in the literature (Boshoff 1942 Pur 19ɔɔ Kyrieleis 1939 Pillat 1933 Graves 193, Donaldson 19,1 Vail Gasteiger 193, Kiss 1934 Culler 1939 Roper 194ɔ Rolandi 191ɔ) In addition Cogan (19ɔ9) claims to have seen about 25 clinical cases The number of described clinical cases is thus relatively small

Histology

Cogan subjected 30 enucleated eyes to histological examination He found the

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SCLERAL PLAQUES

I Incidence and Morphology

BY

M S NORN

A scleral plaque is a transparent superficial well defined scleral area covering a normal white scleral layer The subjacent uvea and the superjacent conjunctiva are normal The plaque is situated in front of an eye muscle attachment

An investigation comprising 1 086 subjects showed a scleral plaque to be a very frequent phenomenon in elderly persons (3 % aged 60 10 % aged 70 and 25 % aged 80 or more)

Plaques are vertically oval (65 %) or circular (27 %) Other shapes are rare They average 2 72 x 1 47 mm in size (the largest one found measured 12 0 x 6 0 mm) Mean distance from the limbus corneae 3 26 mm The plaque increases in size and the distance consequently decreases with increasing age Plaques are usually situated mesially (68 %) and less frequently laterally (19 %) or in front of any of the four horizontal muscular attachments (13 %) In the series under review no plaques were found on the vertical muscles

A possible relationship between plaques and arcus senilis cataract corneal thickness sex incidence and symptomatology is discussed

Key words scleral plaques – preplaques – degeneration (senile hyaline) – transparency – translucency

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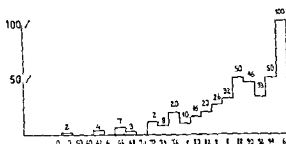


Fig 1
Incidences of scleral plaques in different age groups Abscissa age in 2 year age groups except for patients under 60 Ordinate percentage of subjects with plaques in the age group concerned Subjects totalled 1 0[illegible]6

Table I further shows the incidence of preplaques as found in a previously examined series (Norn 19[illegible]3) As might be expected preplaques come on at an earlier age and the incidence is much higher in all age classes

Fig 1 illustrates the plaques incidence calculated for 2 year age groups after the age of 60 years In general the curve shows a steady rise It is however somewhat irregular owing to the numerically small number of cases within the individual 2 year groups

Sex incidence

The total series comparised [illegible]66 females and 320 males The incidence was 1[illegible]% for the females and [illegible]% for the males This female predominance ($P < 0.0$[illegible]) was not statistically significant within the 10 year age groups

Table I
Incidence of scleral plaques and preplaques dependence on age expressed in percentage of examined subjects (Preplaques from Norn's series 1973)

Ages	< 60	60–69	[illegible]0–[illegible]9	≧ 80
Plaque	1	3	10	2[illegible]
Preplaques	4	30	45	[illegible]4
Numbers	181	[illegible]4	40[illegible]	2[illegible]3

sclera to be neither excavated nor thin within the plaque area but of normal or slightly increased thickness Surprisingly few cells were found within the plaque area compared with the number in the surrounding normal sclera About half of the eyes also showed calcification The collagenous connective tissue fibrils were normal and unlike previous investigators (Culler 1939 Boshoff 1942) Cogan found no hyaline degeneration in his large material

Present Investigations

Within a 2 year period from October 1971 to October 1973 I examined a series of 1 086 subjects with a view to the presence of scleral plaques The subjects were seen as out patients (Eye Clinic Kommunehospitalet and own ophthalmic practice)

The examinations were undertaken in a Haag Streit lamp 900 by direct and indirect illumination The sclera was examined after the patient had made each of the following eye movements upward downward temporal and nasal Plaques if present were outlined on a plate The height and breadth of the plaque and its distance from the limbus corneae were measured to the nearest 0 1 mm by means of a scale built into the slit lamp ocular

Incidence

A total of 111 subjects were found to have scleral plaques It is surprising that within the stated 2 year period I found a greater number of these cases than have been reported in all the literature to date

Age Incidence

The incidence of scleral plaques was found to increase with increasing age The youngest patient was a woman aged 47 years A previous episcleritis had developed into a phlyctenoid formation Her plaque may be secondary to this previous pathological finding The youngest but one was a 62 year old person with no previous eye disease

The incidence of plaques was found to be 3 % for persons aged about 60 years as against [illegible] % for persons aged about 70 years and as high as 25 % for persons aged over 80 years (i e every four persons of this age class were affected)

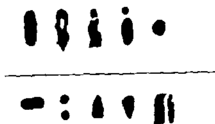


Fig 3
The different morphological types of scleral plaques a total of 21? plaques (See description in text)

The sclera was formerly thought to be thin and excavated at the site of the plaque However using the narrow slit of the slit lamp and high magnification we noticed that the sclera shows practically no flattening The superficial layer of the sclera was seen to be transparent like the cornea with an anterior demarcation in the form of a line following the normal curvature of the sclera

Below this limpid scleral layer is another layer more normal in appearance through which the uvea shines with a greyish tinge

The superjacent conjunctiva seems to be normal and the conjunctival vessels pass unaltered across the plaque

Shape (Fig 3) The scleral plaques are most often *vertically oval* (in 65 %) The oval shape may be more or less regular The outline may be sinuous or even serrated The axis of the oval is generally vertical more rarely slightly oblique and is tangential to the corneal circle

A central sparing was found within the oval plaque in 16 % while in 2 % a transverse total sparing was noticed around a vessel In 1 % a small circular plaque was seen above the oval one

A circular plaque was second in frequency to the oval shape (2? %)

A *horizontally* oval plaque was rare (3 %) as were also two circles one above the other (1 %) and triangles with base down (2 %) or base up (1 %)

Finally a double vertically oval plaque was seen near a muscular attachment in a patient who had undergone recession of the muscle concerned in childhood

In the literature we find likewise that the vertically oval shape is described as being the most frequent (Cogan and others) The oblique tangential oval is mentioned by Graves and the circular plaque by Roper and Boshoff Two circles one above the other have been recorded by Boshoff and Culler

		Plaque	Preplaque
O|	O	29	11
|O	O	13	0
O|	|O	28	44
|O	O|	4	2
O|	O|	1	1
|O|	|O	11	8
|O|	O|	2	3
|O|	|O|	13	31

Fig 2

Sites of scleral plaques in 111 subjects compared with sites of preplaques in 11 subjects (Norn 1973) in per cent

Sites

Fig 2 illustrates the sites of the scleral plaques in the 111 subjects The majority had one mesial (29 %) or two mesial plaques (28 %) while others had four plaques (13 %) or one lateral plaque (13 %)

Predominance of mesial plaques (68 %) was far more frequent than predominance of lateral plaques (19 %)

The sites of preplaques (Norn 1973) are indicated in the same Figure for the sake of comparison In this series mesial predominance (63 %) was likewise much more frequent than lateral (5 %) Scleral plaques were most often found unilaterally and preplaques most often bilaterally off the four horizontal muscles This harmonizes with the hypothesis that preplaques are precursors of plaques Plaques were found only near the insertion of the horizontal muscles and never near vertical muscles or elsewhere on the sclera

The preference of mesial location has been borne out by previous workers (Boshoff 1942 Roper 1945 Cogan 1959)

Scleral plaques in front of vertical muscles have been described in four cases in the literature (Pillat the rectus inf of both eyes Boshoff the rectus inf of one eye Gasteiger one case the rectus inf of one eye and one case the rectus sup of one eye)

Morphology

A total of 216 scleral plaques were detected in the stated 111 patients These plaques were slate grey well defined and separated from the surrounding normal white sclera by a yellowish border

ment The distance from cornea to rectus medialis is 5 5 mm and to rectus lateralis 7 00 mm (Spalteholz 1906) The fact that these figures somewhat exceed the sum of plaque breadth plus limbus distance bears out the suggestion that the plaque rarely extends as far as the muscular attachment

Where the muscle is visible it is seen that the plaque may be situated just off the middle of the attachment or – if fairly small and circular – at the lower corner of the attachment

The literature states that 9 0 mm is the maximum height (Pillat 1933) and ? 5 mm the maximum breadth (Graves 1941)

Symptomatology

Two of the 111 patients with plaques had noticed the plaque themselves

A woman aged 73 years came to the consultating room for medical advice regarding a grey spot on the sclera a 2 0 x 3 0 mm vertically oval mesial plaque of the left eye

A woman aged 62 years thinking she had a foreign body in her right eye had tried to remove the 1 0 x 1 0 mm scleral plaque with a handkerchief!

The remaining 109 subjects had hardly noticed their sceral plaques

One scleral plaque was possibly secondary to episcleritis None of the other cases had a pathological origin

Muscular anomalies were present in three cases However these do not allow us to draw any conclusions regarding possible influence of muscular tension on the development of plaques

Rectus lateralis palsy of left eye with plaque of right eye

Obliquus inferior palsy with two mesial plaques

The previously reported case of recession of the rectus medialis with associated double oval plaque

One patient with corneal dellen had no plaque in the desiccated area of the cornea

Pilot studies

Vital staining of the conjunctiva The part of the conjunctiva covering the plaque was examined after vital staining in a limited number of cases

No signs of epithelial defects were seen (no fluorescein staining) the epithelial cells were neither dead nor degenerate (no rose bengal or tetrazolium staining) and no mucus had been deposited (no alcian blue staining Norn 19 ?) The conjunctiva covering the plaque was normal as was the remaining conjunctiva

Finally Gasteiger in a footnote reported an exceptional case in which a plaque extended uninterrupted from an area in front of a horizontal muscle in a quartocircular course up to the attachment of the rectus superior

Size

Height The heights of the 218 scleral plaques averaged 2 72 mm The mean heights were found to increase considerably with increasing age ranging in the present series from 1 4 mm in the youngest group to 4 2 mm in the oldest group (Table II) The lowest plaque was 0 1 mm high

The highest plaque was 12 0 mm Some were 10 0 mm being in other words as high as the cornea and extending far under the lids at normal lid position

Breadth The mean breadth was considerably smaller than the height being 1 47 mm The breadth was likewise seen to increase steadily with increasing age Minimum breadth was 0 1 mm and maximum 6 0 mm No sex difference was noticed either in breadth or in height

Distance from limbus This was measured from the limbus corneae to the nearest plaque border This distance averaged 3 26 mm The distance was seen to decrease with increasing age the plaque increasing in breadth thus growing in size towards the cornea However there was far less reduction in distance than increase in breadth Thus a growing plaque extends not only out towards the cornea but also back towards the muscular attachment

In some instances the muscular attachment was directly visible through the thin conjunctiva A scleral plaque is situated anteriorly to the muscular attachment with a seam of normal sclera between the plaque and the attach

Table II

Sizes of scleral plaques Dependence on age Mean heights breadths and distances from the limbus corneae expressed in millimetres

Ages	< 70	70–80	80–90	≥ 90	Total
Heights	1 4	1 9	3 0	4 2	2 72
Breadths	1 0	1 2	1 6	1 8	1 47
Limbus distances	3 5	3 4	3 2	3 2	3 26

A scleral plaque is a well defined transparent area in the superficial scleral layer situated in front of an eye muscle attachment The superjacent conjunctiva is normal as is also the subjacent uvea

Unlike a few previous writers I have seen no plaques in front of vertical muscles only in front of horizontal muscles

The above criteria allow us to distinguish a scleral plaque from *scleromalacia perforans* (uncharacteristic location loss of substance) from *deposits* on the sclera (ochronosis argyrosis melanosis and pigment spots characterized by non transparent deposits) and from the precursor called *preplaque* (ill defined localized transparency best seen with the naked eye It is invisible in direct slit lamp light)

The results of a follow up of subjects with scleral plaques undertaken 6 to 12 months after the primary measurement will be reported in a future paper

The follow up examination will show how fast and in what directions plaques can grow and may give some insight into the cause of plaques (muscular tension or desiccation?)

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Scleral or pupillary *transillumination* by scleral lamp or fibre optic light rarely showed any defects in the plaque region The plaques were seen to be situated in the region between the highly pigmented ciliary body and the the less pigmented choroid in other words within a transitional area from dark to translucent layer The plaque in some instances was seen to be co extensive with the translucent choroidal area However in most cases no translucency was noticed under the plaque

This observation differs from the findings by Boshoff and Pillat that scleral or pupillary transillumination may cause the scleral plaques to shine

Arcus senilis This has been graduated arbitrarily from 1 5 Examinations of 22 eyes with plaques and 120 control eyes revealed no statistically significant correlation between the presence of pronounced arcus senilis and scleral plaques

Corneal thickness In seven cases this was measured by a Haag Streit pacho meter in the slit lamp The values were within the normal range

Scleral rigidity This was measured by paired weights in 20 eyes The values were normal

Discussion

The results of the investigation showed that scleral plaques are surprisingly frequent that the incidence increases with increasing age and that such pla ques may be regarded as age determined phenomena

Scleral plaques are of great importance in the differential diagnosis Graves in 1937 reported a case in which the patient s own consultant ophthalmologist had advised enucleation because of suspected sarcoma Graves diagnosis was scleral plaque[1]

Observation through the narrow slit of the slit lamp permitting examination of individual optical sections revealed that the plaque is not due to excavation of the sclera but rather to transparency of the superficial scleral layer A fine line can be seen around the surface of the transparent sclera a line which con tinues uninterrupted along the contour of the surrounding normal sclera

The superjacent conjunctiva is normal The deep scleral layer is likewise normal and whitish while the uveal pigment is visible with a greyish colour showing through the thin whitish scleral layer

According to various writers the subjacent uvea may be transparent If so it should be possible to look directly into the vitreous body through a window consisting of transparent conjunctiva sclera and uvea[1] I have never seen such a window

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EFFECTS OF LOADING OF THE EYE ON THE INTRAOCULAR PRESSURE AND ON THE EPISCLERAL VENOUS PRESSURE

BY

C E T KRAKAU and K WILKE

Loading of the eye with a small weight (2 5 g) for 1–3 *min* caused a depression of the IOP of about 2 to 3 mmHg The pressure in recipient veins likewise decreased to a similar extent The pressure in the conjunctival vessels increased These effects were also observed contralaterally

Key words IOP (intraocular pressure) – vibration tonometry – episcleral venous pressure – pressure regulation

The decrease of the intraocular pressure (IOP) which follows repeated tonometry (Stocker 1955 Moses 1961 Bechrakis 1966) has been studied with reference to some factors which were presumed to be relevant Thus the weight of the tonometer was found to be related to the effect This could almost be avoided by using a sufficiently light instrument (Krakau & Wilke 1971) Sham measurements had no effect on the pressure (Wilke 1972) Measurements on eyes with severe optic nerve damage did not show the effect (Bynke Krakau & Wilke 1972)

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valve The observer controls a joystick by means of which he slowly increases the pre sure of the jet until closure of the vessel is seen This point is most distinctly marked by the arre t of the flow of the blood corpuscle aggregates and other signs of obstruction like regurgitation of blood into the aqueous vein The true venous pressure lies between the levels + and + ++ Its absolute value is not of primary interest to the present study Local anaesthesia was used as in the IOP measurements

Material

All measurements were made on healthy young women aged 20–35 Only people with a recipient vein could be used in the venous pressure series All of them were familiar with the test situation

Experiments and Results

1 Repeated applanation tonometry and venous pressure measurements

The first series was started by measuring the episcleral venous pressure (P_v) both at the + and +++ level The IOP was then measured once a minute

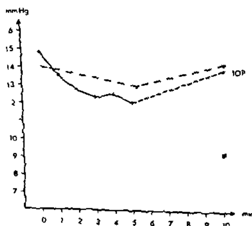


Fig 1

Repeated applanation tonometry and venous pressure measurements (P_v) In each one of three indi duals three series were made The means of these experiments are represented Both the level + and +++ were measured

In attempts to dissect this problem further, the episcleral venous pressure was considered worth studying The aim of the present investigation is to follow the IOP and the venous pressure mainly that of the so called recipient veins in connection with loading the eye with weights of different sizes for various durations

Methods

Measurements of the IOP

The measurements of the IOP were made either by means of Goldmann's applanation tonometer or the vibration tonometer (Krakau 1970) As a rule the instrument was allowed to rest on the cornea for 5–10 sec only when Goldmann's instrument was used the plunger of the vibration tonometer was left for 15–20 sec The load of the latter usually was 0 8 g – Local anaesthesia with Novesin 0 4% was used and all measurements were performed on seated subjects

Loading of the cornea

For loading the cornea we used a plexiglass body similar to that of the Goldmann tonometer The body was hung as a pendulum on a metal rod connected to a horizontal axis mounted on ball bearings The cornea could thus be loaded with different weights A simple hydraulic device made it possible to let the body come into contact with the cornea smoothly and gently

Measurements of the venous pressure

The episcleral and conjunctival venous pressure was measured by means of the instrument described by Krakau Widakowich & Wilke (1973) An air jet is used to compress the vessel and two degrees of effect were used + (= slight deformation of the vessel) or +++ (= total obstruction of the vessel) In every measurement the pressure in the air jet was slowly and continuously increased from a low level until + or +++ was observed The pressures obtained on so called recipient veins (i e an episcleral vein which has just received a large aqueous vein) are denoted by P_{rv} and those on conjunctival veins by P_{cv} The P_{rv} and P_{cv} were always measured in the same recipient or conjunctival vein in the individual eyes

The levels + and +++ were both determined in series 1 (see below) However since these levels run parallel only the level +++ which is the best defined point was determined in the subsequent series We have found that the most reproducible venous pressure values are obtained by letting the pressure change by means of a small reversible D C motor operating on the needle

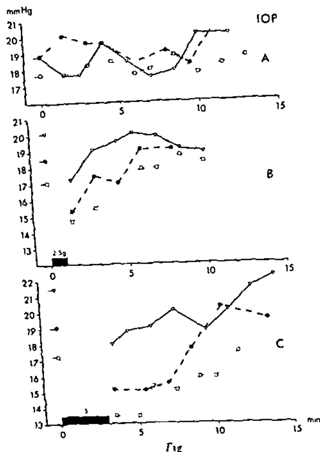


Fig
IOP (vibration tonometry) and loading of the eye (° 5 g) in 3 individuals A control eries B loading for 1 min C loading for 3 min. One curve in series B is the mean of 2 experiments all others are the mean of 3 experiments

Every sub series was repeated two to five times Only one or two series were made on the same day

Before loading of the eye at least two measurements were made and their mean denoted P was used as the initial value

The IOP as recorded by means of the vibration tonometer shows considerable spontaneous variation in some individuals in others the fluctuations are less pronounced These variations can be attributed to errors of adjustment in mea suring and inconstant fixation but there is probably also a true fluctuation of

Table I
Repeated applanation tonometry and P_{rv} measurements

	IOP						+						+++					
	P_0–P_5			P_0–P_{10}			P_0–P_5			P_0–P_{10}			P_0–P_5			P_0–P_{10}		
	r	m	n	r	m	n	r	m	n	r	m	n	r	m	n	r	m	n
G W	2 0	3 3	3	1 0	0 5	2	1 2	1 4	3	0 8	0 3	3	0 8	1 2	3	0 7	0 5	3
B R	1 0	2 7	3	2 0	2 0	3	0 5	0 9	3	0 7	–0 2	3	0 8	1 2	3	0 7	–0 9	3
C H	1 0	2 3	3	2 0	0 7	3	0 0	0 8	3	1 5	–0 8	3	0 7	0 9	3	0 7	–0 5	3

r = range m = mean n = number of experiments
P_0 = first measurement of IOP or P_{rv} (+ +++)
P_5 = measurement after 5 min
P_{10} = measurement after 10 min

for 5 min with the Goldmann applanation tonometer The P_{rv} was then measured again immediately and also after another 5 min Finally the IOP was measured The patient had to remain sitting at the tonometer during all measurements One series a day was made on three different days in three subjects

In complete accordance with previous investigations (cf Wilke 1972) the IOP fell during the repeated tonometry After the sixth measurement the mean decrease of the group was 2 8 mmHg but after a further 5 min the pressure was nearly restored (1 mmHg lower than the initial pressure) (Fig 1 Table I)

In all experiments there was also a slight decrease of the venous pressure (P_{rv} + and + + +) at the second measurement when the IOP was at its minimum At the end of the experiment the venous pressure was restored or was somewhat higher than at the beginning

2 Vibration tonometry and loading of the eye (2 5 g)

In this series of seven subjects the IOP was measured with 1–1½ min interval (except during loading of the eye) by means of the vibration tonometer Every experimentee was subjected to three sub series

a) a control series without loading of the eye
b) loading for 1 min with a weight of 2 5 g on the cornea
c) loading for 3 min with a weight of 2 5 g on the cornea

The measurements of three subjects used also for venous pressure measurements in an analogous series are given in Fig 2

The significance of the decrease has been tested by considering the mean loading effect of an individual and the mean in the corresponding control group as paired observations By means of the t test we find that the difference be tween the loaded and control groups is significant at the 99 8 per cent level for both the 1 and 3 min series

In two subjects with a small pressure response to 2 5 g a load of 5 g was also used in order to find out if a heavier weight gave a greater pressure de crease The effect was much the same as that obtained with 2 5 g (Table II)

3 Measurements of the episcleral recipient vein pressure (P_{rv}) and loading of the eye (Fig 3)

a) Load 2 5 g Three subjects with easily discernible aqueous veins out of series 2 were used P_{rv}(+ + +) was measured once a minute before during and after loading the eye Three sub series corresponding to A (control) B (1 min load ing) and C (3 min loading) of series 2 were made in all subjects The experi ments were repeated two to four times in each individual In the *control series* the pressure fluctuations were slight and no significant trend was found (Fig 4 A Table III)

Loading of the eye for *1 min* gave a pressure decrease The location of the minimum value can be discussed In Table III the second minute was chosen but as seen from Fig 4 B two of the subjects reached their *minimum 1 min* later

Loading for *3 min* gave a pressure decrease the minimum in the third minute being 1 9 to 3 8 mmHg lower than the starting value Judging from the *3 min* loadings the venous pressure restitution seems to be faster than that of the IOP

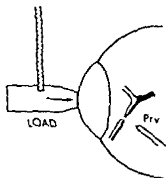


Fig 3
Measurement of P_r and load ng of the eye

A t ph hal

Table II
IOP measurements (vibration tonometer)

		2 5 g ipsilateral load						Control					
		Loading period											
		1 min			3 min								
		P_0–$P_{1\,5}$			P_0–$P_{3\,5}$			P_0–$P_{1\,5}$			P_0–$P_{3\,5}$		
		r	m	n	r	m	n	r	m	n	r	m	n
S H	□ □	0	2 4	2	2 4	3 7	3	3 2	0	3	5 6	–0 5	3
C N J	▽——▽	3 2	2 9	3	1 6	3 5	3	3 2	1 1	3	3 0	1 1	3
G W	●----●	5 6	3 2	3	2 4	4 0	3	2 4	–1 3	3	1 6	–0 8	3
U A		2 4	1 9	3	4 8	6 1	3	2 4	0 5	3	2 4	0 8	3
H W		3 2	2 7	3	2 4	0	3	2 4	0 3	3	2 4	0 3	3
B R		0 8	0 3	3	1 6	1 3	5	2 4	0	3	3 2	0 3	3
G P		0 4	1 8	2	0 8	2 5	3	1 6	–0 3	3	3 2	0 5	3
		5 0 g ipsilateral load											
B R		2 8	0 2	2	1 6	2 1	3						
G P		0 8	1 1	3	2 4	2 1	3						

r = range m = mean n = number of experiments
$P_{1\,5}$ = IOP value after 1 5 min
$P_{3\,5}$ = IOP value after 3 5 min

the intraocular pressure We consider these undulations as a random noise since no significant trend can be traced when several control recordings are superimposed and the mean values at the measuring points are calculated

When a load is placed on the eye a decrease in pressure is recorded We generally find the lowest value in the 1 min series in the first measurement after taking off the load and this pressure has been denoted P_1 Similarly after 3 min of loading the lowest value falls 3 5 min after starting the loading and the pressure at this point is denoted P_3 The quantity we want to test for a significant difference from the control curve is the difference between the initial value and the value at time 1 5 or 3 5 i e P_0–$P_{1\,5}$ and P_0–$P_{3\,5}$ The range and mean of these differences are given in Table II In the control curves the same differences have been measured

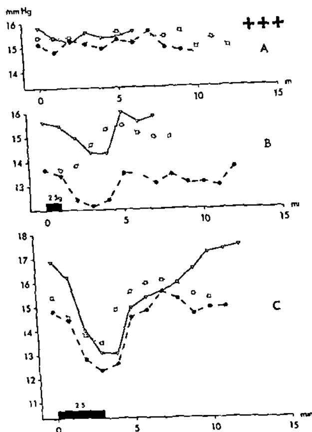


Fig 4
Episcleral venous pressure (P_{ev}) and loading of the eye (2.5 g) in three individuals A control series B loading for 1 min C loading for 3 min Each curve is the mean of 2–4 separate experiments

eye with 17.5 g for 1 min (Fig 5 Table III) A small pressure rise was observed during the loading time followed by a pressure decrease of 1.5–2.2 mmHg The restitution of P_{ev} was slower than that following a loading weight of 2.5 g

A single experiment with 3 min of loading showed a similar curve though of higher amplitude

4 Measurements of P_{ev} during loading of the contralateral eye (2.5 g)

In two subjects the P_{ev}(+ + +) was measured once a minute before during and

Table III

P_{rv} measurements

		2 5 g ipsilateral load						Control					
		Loading period											
		1 min			3 min								
		P_0–P_2			P_0–P_3			P_0–P_2			P_0–P_3		
		r	m	n	r	m	n	r	m	n	r	m	n
S H	□ □	0 6	0 6	2	1 9	1 9	4	1 3	0	2	0 6	0 3	[illegible]
C N J	▽——▽	0	0 6	2	2 5	3 8	2	1 3	0 6	3	0 6	0 9	3
G W	●- - - -●	1 6	1 2	3	1 0	2 5	4	0 3	-0 2	2	0 6	0	[illegible]
		17 5 g ipsilateral load											
G W	●- - - -●	1 3	1 9	3									
B A	■××××■	0 7	2 2	2									
K Å	▼- - - -▼	1 3	1 5	2									
		2 5 g contralateral load											
G W	●- - - -●				0	1 9	2						
S H	□ □				0 2	1 8	2						

r = range m = mean n = number of experiments

P_0 = first value of P_{rv}

$P_2 = P_{rv}$ after 2 min

$P_3 = P_{rv}$ after 3 min

When treating the P_{rv} series in analogy with the IOP series we find that the difference between the means of the 3 min loading series and the control series is significant at the 99 5 per cent level

b) Load 17 5 g Our main purpose in this paper has been to demonstrate the effect of a small load on the eye (2 5 g) on the IOP and venous pressure Although no systematic inventory of the effect of other weights has been performed one sample of the effects of heavier weights can be presented

Special interest attaches to the effect of loading with weights in the range of the tonographic instrument on the P_{rv} Therefore in series of three cases the $P_{rv}(+++)$ was measured once a minute before during and after loading the

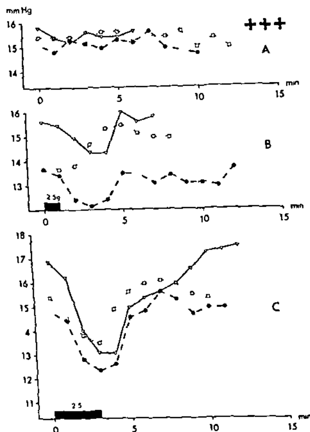


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		Loading period											
		1 min			3 min								
		P_0–P_2			P_0–P_3			P_0–P_2			P_0–P_3		
		r	m	n	r	m	n	r	m	n	r	m	n
S H	□ □	0 6	0 6	2	1 9	1 9	4	1 3	0	2	0 6	0 3	2
C N J	▽——▽	0	0 6	2	2 5	3 8	2	1 3	0 6	3	0 6	0 7	3
G W	●- - - -●	1 6	1 2	3	1 0	2 5	4	0 3	-0 2	2	0 6	0	2
		17 5 g ipsilateral load											
G W	●- - - -●	1 3	1 9	3									
B A	■××××■	0 7	2 2	2									
K A	▼- - - -▼	1 3	1 5	2									
		2 5 g contralateral load											
G W	●- - - -●				0	1 9	2						
S H	□ □				0 2	1 8	2						

r = range m = mean n = number of experiments
P_0 = first value of P_{rv}
P_2 = P_{rv} after 2 min
P_3 = P_{rv} after 3 min

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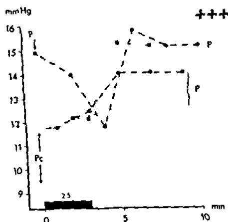


Fig 7

Episcleral venous pressure (P_{ev}) and conjunctival venous pressure (P_{cv}) Loading of the eye (2.5 g) for 3 min The curves represent 2 individuals each measured twice Both P_{ev} and P_c were measured in the same experiment

A mean pressure decrease of 1.8–1.9 mmHg was observed (Fig 6 Table III) This observation gains interest from the fact that the IOP decrease at repeated applanation tonometry is known to involve the contralateral eye

5 Measurements of P_{ev} and P_{cv} during loading of the eye (2.5 g)

In two subjects the P_{ev}(+++) and P_{cv} (+++) were measured alternately once a minute before during and after loading the eye with 2.5 g for 3 min Two series were made on each subject A decrease of P_{ev} of 2.4–3.2 mmHg was observed during the loading time followed by a rapid restitution of P_{ev}(Fig 7) A pressure increase of P_{cv} of 1.9–3.5 mmHg was seen during and after the loading period

6 Photographic recording

Spectacular changes in the episcleral vessels accompany the loading of the eye (Fig 8)

In series A with loading (2.5 g) for 3 min and measurements of P_{ev} a considerable widening of episcleral and recipient vessels (RV and V) became obvious after a loading time of about 2 min. The width of the vessels was normalized some minutes after releasing the load in spite of continued pressure measurements In series B (loading of the contralateral eye) there was a similar but less marked effect No measurements of P_{ev} were made in series B

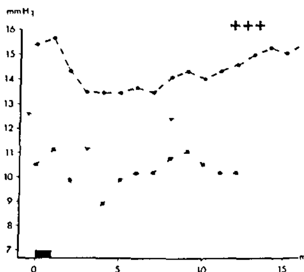


Fig 5

Episcleral venous pressure (P_{ev}) and loading of the eye (17 5 g) for 1 min in three individuals Each curve is the mean of two or three separate experiments

after loading the contralateral eye with 2 5 g for 3 min Two series were made in each subject

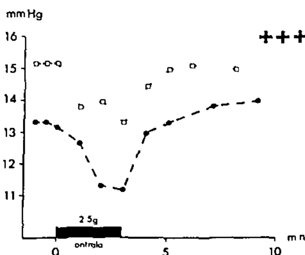


Fig 6

Episcleral venous pressure (P_{ev}) and loading of the contralateral eye (2 5 g) for 3 min 2 individuals each curve is the mean of 2 experiments

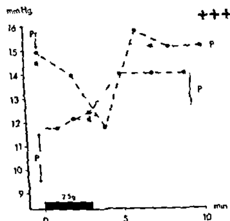


Fig 7

Episcleral venous pressure (P_{rv}) and conjunctival venous pressure (P_{cv}) Loading of the eye (? 5 g) for 3 min The curves represent ? individuals each measured twice Both P_{rv} and P_{cv} were measured in the same experiment

A mean pressure decrease of 1 8–1 9 mmHg was observed (Fig 6 Table III) This observation gains interest from the fact that the IOP decrease at repeated applanation tonometry is known to involve the contralateral eye

5 Measurements of P_{rv} and P during loading of the eye (2.5 g)

In two subjects the P (+++) and P_c (+++) were measured alternately once a minute before during and after loading the eye with 2 5 g for 3 min Two series were made on each subject A decrease of P_{rv} of 2 4–3 2 mmHg was observed during the loading time followed by a rapid restitution of P_{rv}(Fig 7) A pressure increase of P_c of 1 9–3 5 mmHg was seen during and after the loading period

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It may finally be mentioned that in a few cases the experimentee was allowed to sit for 3 min with open eyes without blinking No load was applied No effect on the venous pressure or the width of the veins was observed

Discussion

The original observation of a pressure reduction during repeated applanation tonometry is only to a minor extent attributable to the tonographic effect i e. the squeezing out of fluid from the eye as is evident from the tonographic tables (cf Leydhecker 1960) The same argument is also valid for the decrease obtained with a small weight (say 2 5 g) in series 2

The amount of decrease in these two types of loading varies considerably between individuals but it seems that an individual showing a substantial decrease upon repeated tonometry also shows a marked decrease at constant loading and vice versa It is therefore likely that similar mechanisms come into action in both cases

In attempts to understand these mechanisms we have to consider whether the change of IOP might cause the venous pressure decrease or if the latter might be the primary effect

The time sequences of the P_{rv} and the IOP changes give little hint since they follow each other closely though perhaps the IOP remains depressed longer than the P_{rv} which regains normal values rather quickly after the load has been released

On the other hand we have seen that loading of the eye with a small weight for instance raises the IOP directly but gives no perceptible increase in the venous pressure This is in accordance with the fact that the flow of aqueous is small compared to the amounts of blood flowing through the episcleral vessels

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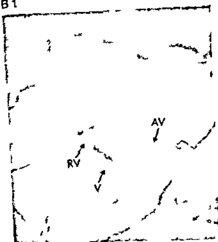
Fig 8

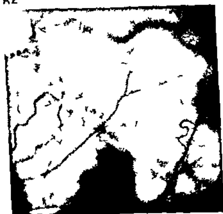
Effect of loading (2 5 g) ipsilaterally (A) and contralaterally (B) A 1 before loading 2 at the end of 3 min loading 3 4 min after taking off the weight P_{rv} was measured every minute

B 1 before loading of the contralateral eye 2 at the end of 3 min loading 3 4 min after the load had been taken off No venous pressure measurements were made in this series

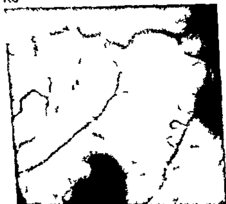
RV recipient vein AV aqueous vein V deep episcleral vein


A1
AV
RV


B1
AV
RV
V


A2


B2


A3


B3

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RV recipient vein AV aqueous vein V deep episcleral vein

of view and study the relations between input and output signals and especially the response of the system to a disturbance of well defined shape A weight was applied to the cornea for some time and the input disturbance is thus simply described by a step function of variable duration and height

As an output signal we may study the IOP or P_{rv} which as we have seen both respond with a decrease to the loading

The input u(t) and the output y(t) of a linear system are connected by the weighting function w(t)

$$y(t) = \int_0^{\infty} u(t-\tau)\, w(\tau)\, d\tau$$

The load has been applied for various periods as summarized in Fig 9 u(t) The response is measured at a series of discrete points and from these values a weighting function can be estimated

Considering first the venous pressure P_{rv} as the output we can calculate an approximate weighting function from the recording of say the 1 min loading experiments The output given by the convolution integral of the calculated weighting function and the input corresponding to 3 min loading and the repeated tonometry series fall well within the error range of the experimental output The magnitude of the depressions in P_{rv} and the time it takes for their minima to appear are in accordance with this description This is schematically shown in Fig 9

This series of effects suggests that the loading time which influences the response in an apparently linear way is the important factor and not for instance the sudden application or removal of the weight

For purely hydrostatic reasons the IOP is immediately increased when the eye is loaded and vice versa For a weight of 2 5 g this change amounts to 1 5–3 mmHg This is in agreement with the value given by Goldmann (1947) who found that the IOP increases by about 1 3 mmHg per g of load In the present set up it was not possible as a routine to follow the IOP during the loading period and this lack of data somewhat obscures the reasoning

However if we admit that the true IOP curves minus the hydrostatic step shaped effect can be smoothly interpolated within the loading period we obtain curves similar to those for the P_{rv} and we can also find a weighting function connecting the output at the different loading periods Whether the re

Conversely an increase of the P_{EV} caused by Valsalva compression or strangulation (Kupfer & Sanderson 1968) is found again in the IOP after a very short lapse.

In terms of flow and resistance it is clear that a reduction of the aqueous production might reduce the IOP but a decrease of a similar magnitude in the P_{EV} could not be expected. Similarly a change of the flow resistance over the trabecular meshwork might reduce the IOP but not the P_{EV}. A change solely in flow and resistance would hardly be sufficient to explain the effect on the P_{EV}.

However both the IOP and the venous pressure effects could easily be achieved by some kind of change in the ocular vessels and the intraocular vascular bed. An arteriolar constriction might reduce the flow inside and outside the eye and also reduce the IOP and the P_{EV} but there is another possibility which should also be contemplated. Generally a somewhat lower pressure is found in the conjunctival than in the recipient veins (Linnér et al 1950 Krakau et al 1973). Loading of the eye induces a moderate increase in the conjunctival pressure as was described by Linner (1954) and by Leith (1963) in connection with tonography. This is also the case in our experiments (Fig 4).

Presuming that the flow from the episcleral recipient vessels will reach the conjunctival vessels a reduction of the flow resistance between these vessels should reduce the P_{EV} and increase the P_{CV} which is quite in accordance with our findings.

We might then expect visible changes in the episcleral vessels. In fact we have noted that during the loading experiments the veins strikingly change their width and blood content. Before accepting the relevance of this observation one has to consider the possibility that these changes might be artefacts induced for instance by the venous pressure measurements. Admittedly some effect on the vessels is seen as a consequence of mere blowing but there are several objections to the view that this slight interference is responsible for the whole effect. Thus vascular changes are observed at loading even when the venous pressure is not measured i e without blowing. We also remember that in spite of continued venous pressure measurements the vascular effect fades away when the load is taken off. An effect on the episcleral vessels is also seen contralaterally.

To the venous pressure measurements it might be objected that the changes are observed in vessels which change their width. Changes of the elasticity of the vessel wall might influence the pressure values. However there is an observation which militates against this suspicion after the load has been taken away a rising pressure is noted in spite of the vessels still being dilated which indicates that the pressure does not depend directly on the width of the vessel.

For further analysis we will approach the system from the „black box" point

fix his eye obliquely – often during the whole period of loading – and this is hard to accomplish for longer periods The loading time has therefore been limited to 3 min

Even the shorter periods of loading give us some hints however After the initial IOP increase at loading the pressure falls and in short time it is near zero or even below the initial pre loading pressure If the error signal were the difference between the ideal pre loading pressure and the actual pressure with a weight on the cornea we would have a rapidly decreasing error But we have seen in discussing the weighting function that the resultant effect agrees well with an error signal of constant height during the loading period It therefore seems unlikely that the pressure itself is the input function It could well be that some other effect such as a strain effect in the wall or a pressure diffe rence between say the IOP and the venous pressure might be responsible for the changes seen Evidently with our present knowledge the anatomical basis for the effect of a load on the eye remains open to speculation

Acknowledgments

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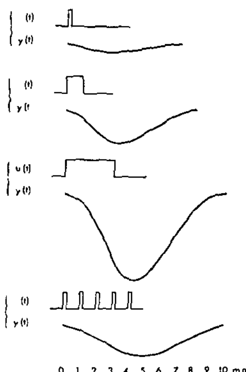


Fig 9
Relation between input functions u(t) (loading of the eye for various lengths of time) and output y(t) (Schematically)

sponse is linearly related to the loading or has not been systematically investigated but it seems clear when applying a load of 17 5 g we have come outside a possible linear range

Is it possible to interpret the pressure decrease which follows a loading of the eye as a sign of a servo regulation of the IOP?

According to definition a servo system is a feed back system that measures the difference between an actual state and a desired state and uses the difference to drive the actual state towards the desired state

The consensual effects of loading an eye could be taken as an indication of the existence of a servo loop The pressure decrease after repeated tonometry does not appear in cases of severe optic nerve damage and this might be interpreted as a severed feed back loop

It would certainly be most interesting to see what happens after so long a period of loading that a steady state might be approximated and the regulation error determined However the experimentee has to sit without blinking and

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REPEATED APPLANATION TONOMETRY IN CAROTID OCCLUSIVE DISEASE

BY

H BYNKE and K WILKE

In nine out of eleven cases of carotid occlusive disease the decrease of IOP on repeated applanation measurements was found to be less in the eye on the same side as the carotid obstruction than in the contralateral eye The results indicate that if there is to be a normal decrease of IOP the blood pressure must be maintained in the ophthalmic artery

Key words Carotis occlusive disease – tonometry repeated

Repeated applanation tonometry once a minute gives a reduction in the intra ocular pressure On an average the pressure decrease amounts to 3 to 4 mmHg in 5 minutes and follows a curve well described by an exponential function (Moses 1961 Bechrakis 1966) The decrease is seen in normal subjects as well as in patients with glaucoma (Bechrakis 1966)

The mechanism behind the phenomenon is obscure The decrease is elicited only by genuine measurements Sham measurements have no effect nor is there any reduction of the response after a great number of series taken in the same subject (Wilke 1973) Although the decrease is not a tonographic effect the weight of the instrument is of importance for its magnitude (Krakau & Wilke 1971) In patients with advanced optic atrophy the decrease has been found to be reduced (Bynke Krakau & Wilke 1972)

As it seemed possible that the phenomenon could be attributed to vascular factors we have examined a group of patients with carotid occlusive disease

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Table I

Eleven cases of carotid occlusive disease

Case No	Sex/age (years)	Angiographic findings	Ipsilateral reduction of corneal pulse amplitude (%)		Decrease of IOP (mmHg)			
			Before surgery	After surgery	Before surgery		After surgery	
					Ipsilat	Contralat	Ipsilat	Contralat
1	f 79	O left	16		1	5		
	f 58	S left	40	10	1	6	6	7
3	m 63	O left+ +S right	−8		1	2		
4	f 63	S left	30		−1	3		
5	m 70	S left	14	7	1	4	2	2
6	m 61	S left	−4		0	4		
7	m 57	S right	27	4	0	2	3	4
8	m 66	O right+ +S left	18		3	3		
9	m 63	S left	11	−24	2	2	4	2
10	m 60	S right+ +S left	0	−9*	1	2	0	3
11	m 67	S right	54		0	3		

O = occlusion and S = stenosis of the international carotid artery

Minus sign means that the pulse amplitude was lowest on the contralateral side

* The *stenosis* was *most* marked on the right side

Material

The material consisted of eleven cases of stenosis or occlusion in the proximal portion of the internal carotid artery (Table I)

The essential symptoms and signs were transient hemiparesis (cases 1 3 5 and 8 11) transient aphasia (cases 1 and 6) homonymous hemianopia (cases 5 and 6) and monocular amblyopic attacks (cases 3 4 7 and 8) The last mentioned symptom occurred as an isolated subjective disturbance in two patients (cases 4 and 7)

There was ocular hypertension without any damage of the optic discs in cases 5 and 6 and in one of them (case 5) the tension was regulated by pilocarpine In cases 1 4 and 7-11 the visual acuity and fields were normal at the time of the ocular examinations

In each case the carotid obstruction was verified by angiographic X ray examinations In case 1 a young woman the unilateral carotid occlusion was a part of the Takayashu syndrome In eight cases the carotid obstruction was unilateral but three patients (cases 3 8 and 10) had bilateral changes

In five patients (cases 2 5 7 9 and 10) the stenosis of one side was treated by surgery (endarterectomy)

Methods

After local anaesthesia with Novesin 0 4 %® IOP was measured with a Goldmann applanation tonometer on the right eye once a minute for four or five minutes (five or six occasions) After an interval of 15 to 30 minutes an identical series of measurements was performed on the left eye For practical reasons it was necessary to restrict the number of measurements in these patients Therefore the effect in the fellow eye on measurements on the first eye (the consensual effect) was studied in only six cases (cf Bynke Krakau & Wilke 1972)

The corneal pulse of both eyes was recorded by a previously described piezo electrical method oculosphygmography (Bynke & Krakau 1964) This examination was performed after the repeated tonometry in eight cases and between two and four hours before the tonometry in the other three cases Eight to twenty eight days after the surgical intervention in cases 2 5 7 9 and 10 both examinations were repeated once or twice

operated upon Δ P of the ipsilateral eye rose to about the same size as in the contralateral eye or was even larger In one single case (no 10) Δ P was 1 mmHg before surgery and 0 after On an average it increased from 1 0 to 3 0 mmHg in these five cases In the contralateral eye Δ P was on an average almost equal before and after the operation i e 3 2 and 3 6 mmHg respectively

The *consensual* Δ P i e the decrease of IOP in one eye on repeated measurements of the fellow eye was found to be lower in the eye on the same side as the most marked carotid obstruction than in the other eye in five of the six patients in whom it was measured

In ten cases the *initial IOP* was between 1 and 5 (av 3 0) mmHg lower in the eye of the side of the most marked obstruction than in the other eye (*Figs 1 and 9*) After surgery it increased on an average by 1 4 mmHg ipsilaterally and remained almost constant contralaterally

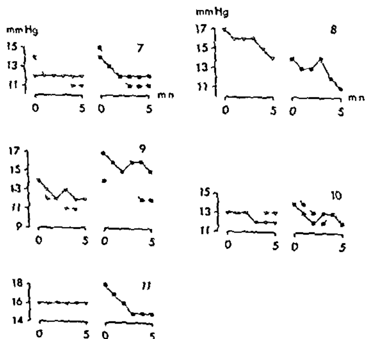


Fig 9

Series I applanation readings from five cases (7 11) of carotid occlusive disease —— curve from the eye on the same side as the obstruction ■—— curves from the contralateral eye Dotted curves and cate readings after endarterectomy

Results

In nine out of the eleven cases the *decrease of IOP* on repeated applanation tonometry i e the difference between the first and last readings (△ P) was found to be smaller in the eye on the same side as the most marked carotid obstruction than in the other eye In the other two cases △ P was equal bilaterally *(Table I Figs 1 and 2)* On an average △ P was 0 8 mmHg ipsilaterally and 3 3 mmHg contralaterally *(Fig 3)* After surgery in four of the five cases

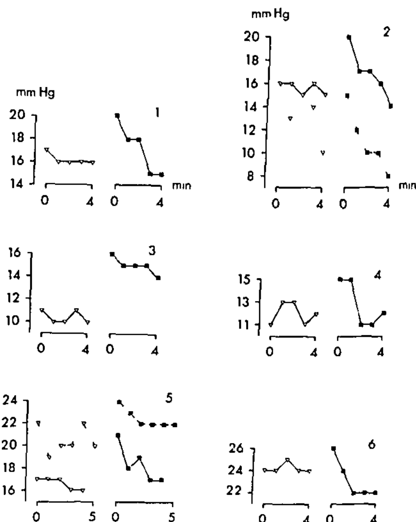


Fig 1

Series of applanation readings from six cases (1 6) of carotid occlusive disease ▽—— curves from the eye on the same side as the obstruction ■—— curves from the contralateral eye Dotted curves indicate readings after endarterectomy

amplitude may be absent or even be reversed in carotid occlusive disease e g if the obstruction is bilateral (Bynke 1968 a)

The artery lumen was restored by surgery in five cases After surgery Δ P was found to have increased in four cases the initial IOP in two and the corneal pulse in all five cases In one case (no 10) however Δ P of the ipsilateral eye was found to be smaller after surgery than before the initial IOP did not change and the corneal pulse increased only a little A repeated postoperative examination gave identical results But this patient was excep tional in other respects too A contralateral hemiparesis appeared at the operation and a postoperative angiography disclosed a marked stenosis in the carotid siphon although the proximal carotid stenosis had been eliminated Therefore we may suppose that the blood supply to the eye was not improved in this case

That *IOP* may be reduced ipsilaterally in carotid obstruction has been observed both in animal experiments (Barany 1946 Davson & Matchett 1951 Kornbluth & Linnér 1955 Bynke 1969) and in man (Chrast & Gottwald 1956 Cristiansson 1962 Bynke 1966 Hørven et al 1971) It is mainly an expres sion of reduced pressure in the intraocular vasculature

It is also well known that the *corneal pulse* is often reduced ipsilaterally in carotid obstruction (Castrén & Lavikainen 1964 Bynke & Krakau 1964 Bron et al 1967 Jensen 1968 Hørven et al 1971) This pulse is produced by rhythmic filling of the uveal vessels (Bynke & Schele 1967) and its dampe ning in carotid obstruction is mainly a manifestation of a reduced pulse pres sure in these vessels But the *mechanism* is somewhat more complex since the pulse amplitude also decreases with the mean IOP (Bynke 1968 b) How ever in carotid obstruction the ocular hypotension is too small – *in the present cases it was only 1 to 5 mmHg* – to have any considerable influence on the reduction of the pulse In animal experiments less than about 10 per cent of the amplitude reduction was attributed to the simultaneous ocular hypotension (Bynke 1969)

The *reduced* Δ *P* in carotid obstruction is as far as we know a new observa tion Although the *material* was small the significance of this phenomenon *is* strongly supported by the fact that Δ P was restored after *surgical treatment*

It might be suspected that eyes which already have a lower *IOP* might react less on *repeated* applanation tonometry than those with a higher IOP But in a study on normal and glaucomatous eyes from subjects in whom there was no reason to suspect any carotid obstruction only a small positive correlation was found between the size of IOP and that of Δ P (Wilke 1972) Further more measurements on ten subjects with side differences of IOP of 2 mmHg or more showed a normal and equal pressure decrease in both eyes (Wilke

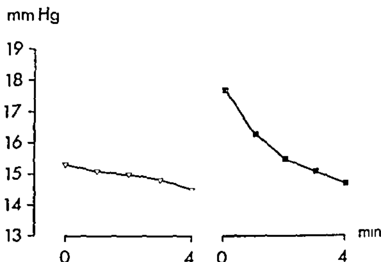


Fig 3

Mean series of applanation readings from eleven cases of carotid occlusive disease ▽— from the eye on the same side as the obstruction ■— from the contralateral eye

In eight cases the *amplitude of the corneal pulse* was between 11 and 54 per cent lower in the eye on the same side as the most marked carotid obstruction than in the other eye *(Table I)* In two cases the amplitude was lower in the contralateral eye but only 8 and 4 per cent respectively and in one case it was equal bilaterally After surgery in the five cases the ipsilateral pulse amplitude was restored more or less and in two cases it became even larger than in the other eye

Discussion

In the majority of the eleven cases Δ P the initial IOP and the amplitude of the corneal pulse were reduced in the eye on the same side as the most marked carotid obstruction

The concordance of the three parameters was not complete in all the cases This discrepancy might be due to methodological errors but also to the fact that the tonometric measurements were made while the patient was seated and the oculosphygmographic examinations had to be performed while the subject was supine There was also a short interval between these examinations and between the tonometric measurements on the right and the left eye Therefore we may suppose that the conditions were not quite the same for all the examinations Furthermore we know that side differences in pulse

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unpublished data) Therefore the slight reduction of the initial IOP in the present cases could not reasonably be the primary cause of the reduced Δ P It is more natural to suppose that both the reduced Δ P and the reduced initial IOP were due to another common factor e g the reduced pressure in the intraocular vessels which is also the mechanism behind the damped corneal pulse

At all events the results indicate that it is a pre requisite for the existence of a normal decrease of IOP on repeated applanation tonometry that the blood pressure is maintained in the ophthalmic artery

It also seems possible that the combination of a reduced initial IOP and a reduced decrease of IOP in one eye will prove to be a valuable sign in the ophthalmic diagnosis of unilateral carotid occlusive disease

Whether the reduced Δ P in advanced optic atrophy (Bynke Krakau & Wilke 1972) is to be similarly explained is still an open question It is true that the consensual Δ P seemed to be different in the two groups of cases but on this point the material is small and further investigation is needed to determine whether the difference is real

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Blindness is a term which is used in reference both to the *whole man* and to the *visual organ* Though they are closely related the two concepts (blind ness of man and blindness of the visual organ) should be clearly distinguish ed in planning social interventions

Blindness of man requires more emphasis on social care in the form of educational economic and social rehabilitation One of the basic tools in ma naging the target population of blind individuals is represented by blind (ness) registers and here the problem of defining blindness arises in a particularly acute way because (for obvious socio economic and socio cultural reasons) the definitions of blindness for the purpose of registration vary very much from country to country[1] It is therefore very hard to arrive at a universal inter nationally agreed *definition of blindness* to be used for registration purposes We feel that this task has to be solved at the national level on the basis of specific socio economic and socio cultural parameters for each country

The various degrees of *blindness of the visual organ* lead eventually to the *blindness of man* When we wish to study the preventive aspects of blindness we have to consider the primary secondary and tertiary preven tion of the blindness of the visual organ This term is equivalent to *ocular mortality* in its final stage whereas less severe stages of ocular (or visual[3]) morbidity can be described by the term *unnecessary loss* of vision [4]

Two aspects of the problem have to be considered here The first is the *concept* of prevention as applied by clinical ophthalmology to single cases and the second is the preventive idea as applied to community health The best way to illustrate the latter is to quote Herman Hilleboe (1971)

Simply stated the preventive idea in community health embraces the concept that s me human ailments environmental hazards to health and health related social

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[1] In s me highly industrialized areas (such as Canada and some of the states in the USA) degree of vision of the order of one tenth of the normal (6/60 or 20 00) falls within the defin tion of bl ndness Various intermediate values are used in diffe ent unt es thus n the Un ted Kingdom the upper limit of vision considered as fall ng within the category of blindness is 3/60 (or approximately 1/ 9 of the normal whil in Egypt and in many other countries it is 1 60)

[2] Visual rgan here is meant in the physiological sense encompassing in addition t th *Bulb s* which is the visual organ in the anatomical sense also the optic n r e and the visual pathway

[3] Th te m of visual *morbidity* has been recently proposed by H T Wyatt to d gnate all ocular m rbidity *accompanied* by visual impairment.

[4] The term unnecessary is widely used in the *anglo* saxon literature and stands f r preventable loss of vis on

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PREVENTION OF »BLINDNESS«

Potentialities of a Systems Analysis Approach

BY

B NIŽETIĆ

A multitude of activities are carried out under the label of prevention of blindness A definition of this term and of related concepts is discussed with the aim of rationalizing and bringing more precision into the planning and evaluation of programmes in this field
Emphasis is placed on the importance of an appropriate eye health information system of a systems analysis methodology for the rational planning of eye health services and of the necessary manpower in the field of public health ophthalmology A definition of this field is presented and illustrated by a conceptual systems model

Key words public health ophthalmology – eye health planning – visual impairment – blindness – systems analysis – prevention – eye health information system

1 Definition of terms

A multitude of activities and programmes are carried out by governmental intergovernmental and nongovernmental agencies throughout the world under the label of "prevention of blindness Both these terms sometimes lead to a certain amount of confusion and it is felt that a clearer definition would bring more precision into the planning and evaluation of programmes in this field

This article was submitted as working paper for a WHO Study Group on Prevention of Blindness convened in Geneva from 6 to 10 November 1972

registered individuals while the concept of *blindness of the visual organ* (or loss of vision or visual impairment) will cover a much larger group of potential or actual ophthalmolo patients the size of which will again depend on the choice of type of prevention This choice will also condition the type of activity to be carried out

Basically population A will be the target for preventive and public health ophthalmological activities while populations B and C are classically taken care of by institutionalized out and in patient eye departments

3 Need for comprehensive eye health planning and evaluation

Considering that almost all eye health programmes include health educational components as well as aspects of training for the necessary manpower and acquisition of new knowledge through investigative procedures without forgetting organization and reorganization of eye health services we realize that instead of speaking of preventive programmes related to blindness the use of the comprehensive concept of the *public health approach to problems of eye health* becomes much more logical

It is not unusual for far reaching interventions to be applied without planning without examining alternatives and without exploring for side effects or long term consequences It is clear that in many situations massive interventions cannot be avoided but it is equally clear that they must be chosen with discretion to ensure safer simpler more effective and/or more economical alternatives If deliberate choices are not made then the choice is often determined arbitrarily by ability to pay by geography or by the publicity afforded a particular consumer of services

We know today that even single agent caused *blinding conditions* like trachoma onchocerciasis and xerophtalmia present multifacet issues in the investigation planning and service providing phase

An ecologically – even more than epidemiologically – oriented approach allowing the avoidance of programmatic pitfalls would require analysis of information in terms of probable or potential eye disease relationship so that alternative points for effective intervention can be selected and sharply specified services can hit the target

4 Systems analysis approach

The most significant activity in the technical effort of planning concerns the systematic analysis of the problem and this involves two major steps

First comes the analysis of the clients (victims or persons at high risk) Next comes the analysis of the intervention or service providing systems in terms

problems can be prevented from occurring This is the real meaning of *primary prevention* requiring no explanation or definition The other interface of the preventive idea is that the ailment hazard or social problem is already present in the individual family or community and in some instances can be *prevented from progressing* This is the true meaning of *secondary prevention* Thus prevention of occurrence and of progression are the key stones in the arch that supports the foundation of those programmes activities and techniques that we use to promote maintain and improve community health

Accordingly a unified concept of prevention to the health worker embodies the application of knowledge and processes acquired from the medical social and environmental disciplines for the purposes of preventing the occurrence or progression of disease defects disabilities[1] and injuries In preventive medicine it is the individual sick or well alike who is the focus of attention In public health the focus is on groups of individuals formed into a community whose members face common health problems among whom an organized community effort is essential for their resolutions

2 Definition of target population for eye health programmes

The choice of target populations will depend greatly on our choice of terms (blindness of man/blindness (visual impairment) of the organ) as well as well defined primary secondary or tertiary prevention

Fig 1 shows that the term *blindness of man* will require the emphasis to be put on social care and will limit the target population to registrable and/or

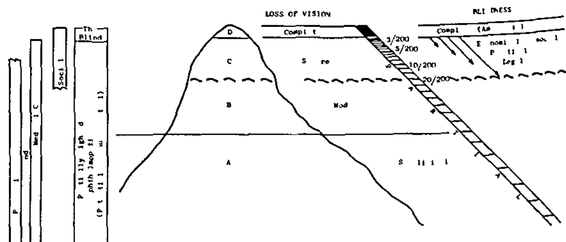


Fig 1
Iceberg showing the interrelationship between blindness loss of vision and different aspects of care delivery

1 Disability limitation is labelled by some authors as tertiary prevention Leavell H R & Clark E G (1958)

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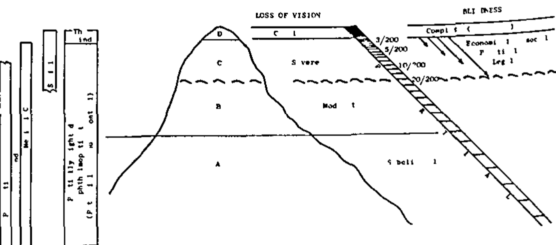


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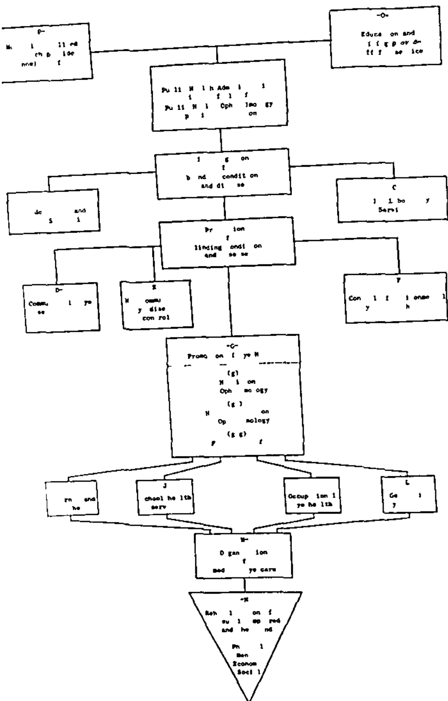


Fig 9

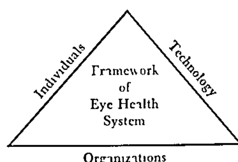
A systems model of public health ophthalmology illustrating its unity and the interrelationship of its constituent activities

of how proposed new condition – based interventions (or redesigned old ones) can be achieved

Systems analysis (based on Von Bertalanffy s General Systems Theory) offers a powerful tool in this respect being a selection of elements relation ships and procedures to achieve a specific purpose (Van Courthare jr 1967) like the one of preventing unnecessary visual impairment and promoting full eye health

The individual his health and the mechanisms influencing his health are readily perceived as *natural systems* and any perceived system can be a target for systematic scrutiny Several health systems perceptions have already been defined structured analysed and occasionally manipulated (Sheldon et al 1970)

An eye-health system can also be visualized as a network of eye health oriented individuals technologies and organizations



Individuals in this framework are represented by eye health professionals allied health personnel patients and public Each individual or group of individuals may have a different image or conceptual framework of the system depending upon vantage point and encounter with the *concrete system* which is the real natural object of scientific study These conceptual frameworks are employed to organize individual or group decision making and future action

A conceptual system perceived by an eye health professional may be pre sented in the form of a prose statement or a graphical model or even in the form of a computer simulation of the system A conceptual system is in some way isomorphic to a system which can be studied as a subject of science – either a *concrete system* or an abstracted system What is meant by *progress in science* is the improvement of conceptual systems so that they gradually be come more isomorphic to concrete or abstracted systems Then they facilitate better understanding and more accurate prediction of the latter type of systems

Fig 2 shows a proposed conceptual model of public health ophthalmology (PHO)[1] illustrating its unity and the interrelationship of its constituent activities (Nižetić 1973)

A continuing process of administrative and technical evaluation as illustrated in Fig 3 (Nizetić 1973) should provide the necessary feedback for the improvement and revision of activities related to the PHO system

Questions could be raised about the general applicability of innovative approaches to eye health planning A deeper look at the issuses we believe will reveal that geographical location or climate have little to do with the basic processes of eye health programme planning The geographical factors may govern the final distribution of effort but a valid approach to programme planning will work anywhere as long as effective means are available for deriving the requisite information about eye health status

5 **Eye-health information systems**

The above considerations lead us to mention a few of the factors related to the development of a reliable and efficient *eye health information system* considered as a prerequisite for the planning of eye health programmes and projects (see Fig 3) It should be understood that the under mentioned factors represent an ideal set the degree of technical sophistication of which has to be reviewed against the background and development stage of each particular country

1 Characteristics of a *data base* containing comprehensive medical eye health and social information on the population receiving eye health care

2 Modes of input based on acts of provision of eye health care to *individual* persons

3 Modes of input involving services provided by all professional and allied categories of the *eye health care team* (ophthalmologists general practitioners orthoptists optometrists etc)

4 Modes of input linking all fixed field and contract care activities

5 An on line system containing medical information from *active files* which is instantly retrievable in problem oriented format to guide *episodic* medical care for individuals

6 Automated output of routine medical and health reports

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[1] PHO is defined as the body of knowledge encompassing the comprehensive community approach to the promotion of eye *health* and particularly to the prevention of disability due to visual impairment and blindness The basic tools for research and practice in this field in addition to *clinical* knowledge are epidemiological and modern management procedures

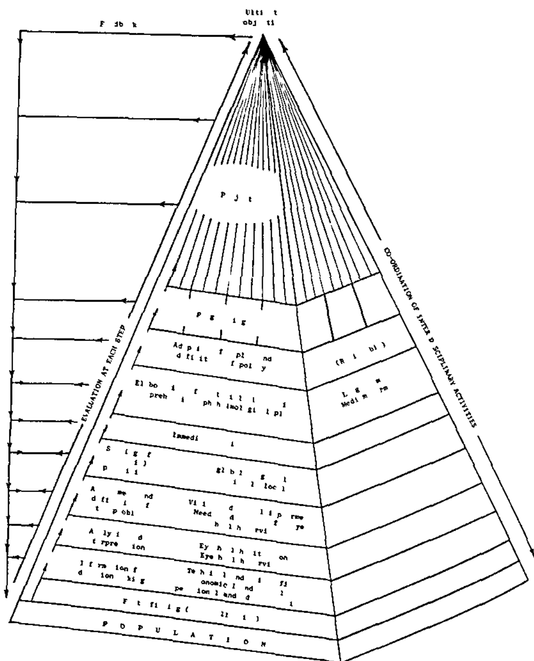


Fig 3
Conceptual model of public health administrative activities applied to ophthalmology

delivery systems used in extended eye care facilities for specific groups of population (pre school and school children) productive age groups (occupational eye health problems) the aged (ophthalmogeriatric problems)

It should however be mentioned that each of these organizational sub systems has to be closely related to the larger general public health systems of each country As a consequence an efficient co ordination with all services involved and careful long term planning for eye health services are essential It should be pointed out also that a substantial amount of research and new knowledge is needed in this field To meet the requirement of comprehensiveness the systems approach would seem to be the only rational one As stated by White (1971)

> To qualify as a system any arrangement for the provision of personal health services should offer a full range and all levels of patient care for defined populations have a well defined organizat on that is fully accountable and acceptable records communications and transportation systems Requirements for the development of a system include leadership capital cash flow and a clear focus of control and responsibility Standards would need to be promulgated with respect to organization and staffing utilization and quality of care Contractual negotiations between the four parties nvolved – consumers physicians fiscal intermediaries and organizers of services and systems – should encourage diversity competition prudent and responsible use of the system and sound quality

8 Concluding remarks

The systems approach can be considered from two extreme positions with a wide range in between at one end of the spectrum the important criterion is the sta ement of a problem while at the other is the need for highly sophisticated mathematical solutions to the system s alternatives which may have been suggested

The traditional techniques for isolating and solving problems are not appropriate owing to the magnitude or complexity of the issues in the field of public health ophthalmology Such techniques as operations research do not view the total system Professional judgement without knowledge of the overall system does not reduce the uncertainties and risks involved Consequently the general concept of systems analysis seems to be essential to a logical development of programmes in the field of prevention of blindness

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7 Retrieval capabilities of wide latitude based on elective use of a large number of conditional retrieval parameters capable of providing listed tabulated or analysed data in a wide variety of formats

It seems obvious that this last point is the most important as far as obtaining eye health status information in a form providing direct input into programme planning is concerned

6 Manpower

The delivery of comprehensive eye health care to growing populations requires an appropriate quantity and quality of manpower The necessity of adequate health manpower planning is recognized in most areas of the world (Methods to achieve this are described in a recent WHO publication (1969)) The relative shortage of highly specialized health personnel and the necessity for an intra disciplinary approach to the prevention of loss of vision make obvious the need for a structural analysis of the situation and a search for new solutions One of the proposed solutions is the increasing use of allied health personnel (nurses ophthalmic assistants ophthalmic technicians ophthalmic technologists orthoptists and pleioptists as well as the optometrists) working as a team in the delivery of complete eye health care under the direction and supervision of the ophthalmologist

Many still controversial and as yet unsolved problems exist in this field Before the interrelationship and responsibilities of different members of such a team are finally settled and the standards of the educational process for each category are fixed further studies on education in the ophthalmological field are essential These studies to be comparative should possibly be based on a universal conceptual model arrived at by systems analysis (Nižetić 1973 Grimes 1971)

7 Organization of eye health services

The conception and planning for continous eye care from prevention to rehabilitation is hindered by several legal governmental and technical difficulties

In general different forms of eye care are offered to the public in (a) hospitals (eye departments in general hospitals autonomous specialized eye hospitals or institutions eye departments in universities and other teaching hospitals) and (b) extended care facilities which are administratively and technically more or less independent of the above mentioned institutions In addition communicable eye diseases control programmes (integrated in various degrees into basic health services) are performed in various countries

It is beyond the scope of this paper to go into the details of the different

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SEX LINKED ESSENTIAL NYCTALOPIA IN A NORWEGIAN FAMILY

BY

KJELL SYVERSEN

Sex linked essential nyctalopia has been demonstrated in eight male members of a Norwegian family with a known occurrence of night blindness The study includes six generations consisting of 65 persons of both sexes All the persons with nyctalopia also had excessive myopia and reduced visual acuity One female member had an incomplete picture of the syndrom but no nyctalopia It is suggested that she represents an incomplete genetic penetrance and a possible gene carrier

Key words night blindness – nyctalopia – sex linked inheritance – incomplete genetic penetrance

Essential nyctalopia is a distinct clinical entity with a clinical picture differing from the tapetoretinal degenerations of the fundus (Cunier 1838) In the ophthalmological literature three different modes of inheritance are described a dominant form (Nettleship 190) an autosomal recessive form (Gassler 1925) and a sex linked recessive form (Nettleship 1912) All three types are congenital and non progressive The dominant form shows no ocular disability apart from night blindness The autosomal and the sex linked recessive forms are

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both associated with myopia and differ very little except in their mode of inheritance Fundus changes corresponding to the degree of myopia may be found but there are no other objective progressive lesions Each genotype has a distinct homogenous picture and shows no associated extra ocular signs

The sex linked, essential nyctalopia is associated with myopia of 6 13 dioptres and usually with an oblique astigmatismus of 1 5 4 dioptres Optimally corrected visual acuity is seldom better than 6/12 The visual fields are normal The nyctalopia is stationary and remains unchanged throughout life Maximum dark adaption is obtained within 15 min Myopic conus is frequently observed and nystagmus is an inconstant feature Sometimes there may be a slight hyperpigmentation of the fundus but not of the retinitis pigmentosa type (Varelmann 1925) The diagnosis is usually obvious from the clinical pattern In addition a typical ERG has been noted (Schubert & Bornschein 1952)

From Denmark two pedigrees with dominant essential nyctalopia have been published (Rambusch 1909) Björk & Karpe (1951) described extinguished ERG in two sporadic cases of presumed hereditary nyctalopia Reports of autosomal recessive or sex linked recessive nyctalopia have not been published in Scandinavia This work has been done in order to present a pedigree of sex linked essential nyctalopia by examining a Norwegian family with a known history of diminished night vision among several male members

Material and Methods

The material consists of 56 members of a Norwegian family originating from the region of Trondheim with branches in Bergen and Oslo The work was initiated by information from a female patient with rosacea keratitis She reported that her grandfather was blind after sundown but had normal vision during the day Some of her male relatives had inherited grandfather s eyes

From this family 24 members have been examined by the author The examination included routine ophthalmological examination with slit lamp direct ophthalmoscopy visual acuity and dark adaption test with Goldmann Weeker s adaptometer Patients with pathological dark adaption or subnormal vision were tested with Haag Streit Goldmann perimeter and Ischihara s charts Exact information concerning two male members of the family with probable nyctalopia was not obtained as both were dead In addition written inquiries were made concerning 30 family members of both sexes with special stress on the occurrence of defect dark vision

Results

Data were obtained on 56 family members concerning eye di eases and night vision The examinations revealed six male members with nyctalopia myopia from -6 to -16 D nystagmus and myopic fundus None of them had better than 6/6 6 visual acuity on the best eye Five had oblique astigmatismus corrected with cylinder - 2 5 to -3 5 Concommitant squint was demonstrated in five out of these six males

There are reliable reports of two more males with nyctalopia and poor vi s on The eldest (I/1) was an active politician and in his part of the country it was well known that he always had to be guided from political meetings by one of his children because he could not see in the dark The other one (V/2) was killed by a car at 6 years of age when he was playing in the street His mother reported that he used to hold his toys close to his eyes and in the dark his vision was very poor He also had restless eyes

Nyctalopia was not found in any of the females Except for one single case myopia and astigmatismus could not be found in the non nyctalopic family members This one case was a woman 22 years old (V/3) with a slight myopia (1 5 o u) and astigmatismus 0 0 5 that could not be corrected to more than 6 1° with glasses contact lenses or pinhole She had no myopic conus nystagmus or nyctalopia

There was no consanguinity in any of the persons included in the material

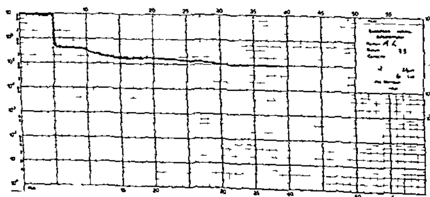


Fig 1

Typi al dark adaptation curve of a patient with essential sex linked nyctalopia

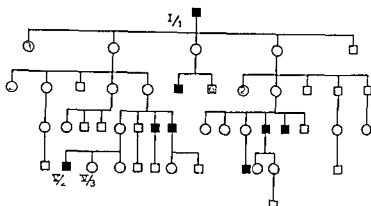


Fig 2
Sex linked essential nyctalopia in a Norwegian family

Discussion

The examinations have shown six male members of a family presenting the typical picture of essential sex linked nyctalopia In addition there are reliable reports of two male members with nyctalopia

One female member of the family had some of the characteristics of the syndrome but no nyctalopia She may represent an incomplete genetic penetrance of the syndrome and a possible carrier state She has however no children and the carrier state cannot be proved There were no similar findings in any of the examined female carriers I have not seen reports of abortive cases of essential sex linked nyctalopia but Nettleship (1912) found the complete picture in a female member of an affected family

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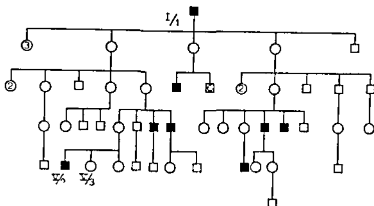


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THE EXUDATIVE LESIONS IN DIABETIC RETINOPATHY WITH SPECIAL REGARD TO THE HARD EXUDATE

BY

L YANKO H UNGAR and I C MICHAELSON

Histochemical techniques were used to study the hard exudates of diabetic retinopathy Digested flat retinal preparations were examined for correlation between the frequency of these exudates and the degree of retinal capillary changes The hard exudates in the retinae appear to be composed of a mixture of glycoproteins lipoproteins and phospholipids with an admixture of neutral fats Study of flat preparations showed that there was a correlation between the density of the exudates and the degree of capillary changes There is no histological confirmation in the few circinate areas examined that the capillary lesion is predominantly in the central area of the circinate

Key words diabetes mellitus – diabetic retinopathy – retinal hard exudates

Exudative lesions are common extravascular components of diabetic retinopathy The exudate *clinically described as being of hard or waxy appearance*

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is usually situated in the outer plexiform layer while the soft type exudate represents a localized area of swelling in the nerve fiber layer of the retina Whereas the pathology and pathogenesis of the soft exudate have largely been elucidated (Ashton 1970) there is still a lack of agreement regarding the nature and source of the hard exudate These deposits are regarded by some investigators as a break down product of degenerated retinal nerve elements (Wolter 1961 Bloodworth 1962) However fluorescent angiographic evidence suggested that the exudates may arise from blood constituents following leakage from defective retinal vessels (Maumenee 1968 Kohner & Dollery 1970)

The aim of this study was to provide additional information regarding the source and nature of this exudate The approach used was histochemical The correlation between the presence of these deposits and retinal capillary changes was also investigated The findings add further support to the *theory that the* exudates may arise from blood constituents

Materials and Methods

The material for this study consisted of ten eyes removed at autopsies *from five* patients with long standing diabetes varying from 1? to 18 years Following fixation in 4 formaldehyde the globes were divided coronally *through the ora serrata* and the retinae were examined with a stereomicroscope Following this examination the globes were divided sagitally into two halves through the optic disk and two *different* approaches were followed

(a) *Flat retina preparation* A few circular areas of retina each one containing a ring of exudates were cut out with a trephine and were digested according *to* Kuwabara & Cogan's method (1960) Additional areas of retina were *subjected to the* same digestive procedure These were taken from eyes *with extensive* hard exudates not arranged ring wise and from eyes with only few *dispersed* exudates The digested retina was mounted on a *slide dried* and stained with the periodic acid Schiff procedure (PAS) and hematoxylin

(b) *Cross sectional histology* Five eyes were treated by chromation (Ciaccio's method) to improve their *resistance to fat solvents* *These* were examined *after* paraffin embedding or frozen sectioning The remaining five *eyes* were embedded in paraffin without chromation Serial paraffin sections *from* all ten eyes were stained with hematoxylin and eosin Gram Weigert's method for *fibrin and* the Mallory phosphotungstic acid hematoxylin (PTAH) method *Appropriate* histochemical staining techniques as described in Table I were used *Frozen* sections were stained with oil red 0

Table I

Histological and histochemical reactions within hard exudates of diabetic retinopathy

Staining procedure	No of eyes examined	Reactions
I Histological empiric stains		
1 Hematoxylin eosin	10	Positive
2 Weigert fibrin	10	Positive
3 Phosphotungstic acid hematoxylin	10	Positive
II Histochemical stains for		
a Carbohydrates		
1 Periodic acid Schiff (PAS)	10	Positive
2 Periodic acid Schiff after diastase	5	Positive
3 Alcian blue pH 2 5	10	Negative
4 Rinehard and Abul Haj colloidal iron	10	Negative
b Lipids		
1 Sudan black B following chromation (Ciaccio s method)	5	Positive
2 Luxol fast blue	10	Positive
3 Luxol fast blue after pyridine*	10	Positive
4 Luxol fast blue after chloroform methanol*	10	Positive
5 Luxol fast blue after ether*	10	Positive
6 Luxol fast blue and PAS	10	Positive
7 Oil red 0	5	Positive
c Protein		
1 Ninhydrin Schiff	5	Positive

* Deparaffinized slides were kept immersed in the solvents for 18 24 hours at 37° C

Results

Stereomicroscopic examination

The retina of eight eyes showed microaneurysms hemorrhages many hard exudates and occasionally moderate sized soft exudates in the posterior retina The hard exudates were of various sizes and often arranged in circular shapes (circinate patterns) which sometimes surrounded the macular area (Fig 1) In the remaining two eyes there were only a few microaneurysms and few dispersed hard exudates



Fig 1
Postmortem appearance of a large ring exudate in diabetic retinopathy

Flat retina microscopy

In digested flat preparations the retinal capillaries show microaneurysms caliber variation strand formation and loss of nuclei Most of the microaneurysms are of a saccular type and of various diameters Alongside the microaneurysms there are caliber variations of the capillaries due to dilatation and decrease of diameter and capillary strand formation A prominent lesion in most of the capillaries is a loss of either mural or endothelial cells or of both types when the vessels are recognizable only by the basement membrane sheath

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4 Luxol fast blue after chloroform methanol*	10	Positive
5 Luxol fast blue after ether*	10	Positive
6 Luxol fast blue and PAS	10	Positive
7 Oil red 0	5	Positive
c Protein		
1 Ninhydrin Schiff	5	Positive

* Deparaffinized slides were kept immersed in the solvents for 18 24 hours at 3 ° C.

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Fig 3
Section of retina showing sudanophilic substances within the hard exudates (Sudan black B × 960)

Gram Weigert's fibrin stain shows a diffuse bluish violet color in many hard exudates Sections stained with Mallory's phosphotungstic acid hematoxylin method (PTAH) also reveal blue violet tint areas in some exudates

In order to identify the presence of carbohydrate components sections were stained with the periodic acid Schiff (PAS) procedure alcian blue at pH 2 5 and colloidal iron (Rinehart Abul Haj) The hard exudates stain bluish red with PAS and resist malt diastase digestion The exudates fail to stain with alcian blue and with colloidal iron methods indicating the absence of mucopolysaccharides

Following chromation and staining with Sudan black B (Lillie 1965) large and granular greyish black deposits are found in many hard exudates of the eyes examined (Fig 3) Most of the exudates show non homogeneous positive staining with luxol fast blue (Fig 4) Exposure of sections to various lipid



Fig 2
Digested flat retina showing microaneurysms caliber variations of the capillaries residual capillary strand formation and loss of cells (PAS and hematoxylin x 105)

(Fig 2) These vascular changes are present predominantly in the *posterior* segment of the retina corresponding with the areas where the hard exudates and hemorrhages were observed under the stereomicroscope

Cross sectional histology

Exudates are present in all specimens examined and are located mainly in the outer plexiform layer of the retina Sometimes large round cells with small nuclei are present in or around the exudate "gitter cells Occasionally red blood cells are seen in or about the exudate In addition there are often varying combinations of other extravascular changes such as cytoid bodies consisting of foci of coagulative necrosis in the nerve fiber layer retinal edema with a *predilection for the outer plexiform layer* or *hemorrhages* affecting all layers of the retina Cross sectioned microaneurysms are often observed

The relevant staining characteristics of the hard exudates are summarized in Table I Most of the exudates stain various shades of pink with hematoxylin and eosin and in a *few of them minute empty spaces like vacuoles are seen*



Fig 5
Section of retina showing different staining within the hard exudates following combined procedure of PAS and luxol fast blue methods (x 105)

of non carbohydrate containing substances such as unsaturated lipids and phospholipids If one takes into account that in paraffin sections lipid substances are either no longer present or present in such small quantities that they do not react and if glycogen has been removed by diastase digestion only one class of materials may then give a positive PAS reaction These are considered to be glycoproteins

The presence of fatty substances can be demonstrated by various methods Neutral fats in the hard exudates were shown in frozen sections stained with oil red O In paraffin sections in which the lipids soluble in alcohol and xylene had been removed during the embedding procedure a considerable amount of lipid containing substances staining with luxol fast blue remained within the hard exudates In sections treated with the double procedure of the PAS and the luxol fast blue methods materials staining with either procedure appeared sharply separated (Fig 5) This observation in addition to the fact that the fast blue staining substance was found to be insoluble in ether pyridine or methanol chloroform mixture suggests the presence of lipoproteins (Pearce 1960) This is supported by the positive staining with the Ninhydrine Schiff

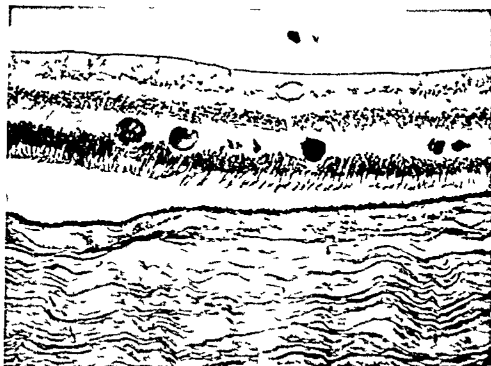


Fig 4
Section of retina showing staining of hard exudates with luxol fast blue (x 105)

solvents (pyridine chloroform methanol ether) did not change the results of subsequent staining with luxol fast blue Following combined staining with luxol fast blue and PAS the hard exudates show a reddish purple stain as well as contiguous areas of blue staining (Fig 5) The Ninhydrine Schiff method reveals traces of violet staining in some of the hard exudates

In frozen sections the hard exudates stain brilliant red with oil red 0

Discussion

The results indicate that hard exudates in diabetic retinopathy are of a non homogeneous composition throughout Carbohydrate components were present in sufficient concentration to give a positive reaction with PAS The work of Leblond et al (1957) greatly clarified the status of PAS reaction in histochemistry The main groups of substances which may be expected to give a positive PAS reaction include polysaccharides glycoproteins glycolipids and a group

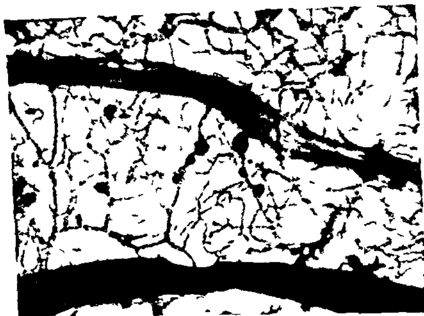


Fig 6

Digested flat retina showing microaneurysms caliber variations of the capillaries residual capillary strand formation and loss of cells within the center of a circinate exudate (PAS and hematoxylin x 105)

However there seems to be no special concentration of these capillary changes within the centers of the circinate exudate (Fig 6) as suggested by Maumenee (1968) and Kohner & Dollery (1970) in their clinical studies This may be due to the fact that the circinate lesions were old and in the process of being absorbed

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method which reveals the presence of protein in the hard exudates (Yasuma & Ichikawa 1953) Obviously the positive Ninhydrine Schiff reaction may also be related to the presence of glycoproteins as was indicated before Paraffin sections of previously chromated tissues showed staining with Sudan black B within the hard exudates The sudanophilic substances which remain insoluble during paraffin embedding in Ciaccio s methods are often regarded as phospholipids (Pearce 1968) although this method has not been entirely accepted

Staining properties of the retinal hard exudates in diabetes have not been extensively studied previously Toussaint et al (1962) found the staining characteristics with oesin and PAS of the exudative lesions to be similar to those of subretinal fluid and therefore considered these exudates to be of seral origin Nevertheless Bloodworth (1964) in a histochemical and electron microscopic study suggested that at least part of these exudates may derive from degenerated retinal tissue In the present study the hard exudates appear to be composed of a mixture of glycoproteins lipoproteins and phospholipids with an admixture of neutral fats The staining reactions showed apparent separation of these substances However this may be the result of fixation procedures rather than a true state of separation in life The occasionally observed positive staining with the empiric fibrin methods (Weigert s & Mallory s PTAH) nowhere revealed fibrillar structure resembling true fibrin This is not consistent with findings by Bloodworth (1967) who in an electron microscopic study noted the presence of fibrin in the hard exudates

Some of the exudates showed large round cells with small nuclei and granular corpuscles dispersed into an abundant vacuolated cytoplasm referred to as "gitter cells These cells with their presumably scavenger function have largely been described in diabetes (Wolter 1961) and in other forms of exudative retinopathy such as Coat s disease (Marshall & Michaelson 1933) and in hypertension (Leishman 1957)

From the work of Cogan et al (1961) de Oliveira (1966) and Bloodworth & Engerman (1971) we know that the retinal capillary changes in diabetes include the loss of intramural pericytes or the loss of endothelial cells or loss of both types of cells In the latter case empty basement membrane sheets or often capillary strands of approximately one third the size of normal capillaries are found in the digested flat retina Alongside these degenerative changes a variable number of microaneurysms are found in the affected retinae The dynamic process of circinate formation has through fluorescein studies been explained by Maumenee (1968) and Kohner & Dollery (1970) on the basis of a continuing central capillary lesion

In the digested flat preparations of this study changes similar to those described by Cogan (1961) and Bloodworth & Engerman (1971) are observed

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QUANTITATION OF ALPHA CRYSTALLIN IN HUMAN AQUEOUS HUMOR BY RADIOIMMUNOASSAY

BY

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A method for quantitation of alpha crystallin in individual samples of human aqueous humor by radioimmunoassay is described The concentration of alpha crystallin in aqueous humor from 48 eyes with cataract and three eyes with clear lenses was at or below 0 006 μg/ml Two samples from eyes with heterochromic cataract contained 0 011 and 0 019 μg/ml respectively Alpha crystallin is probably not among those lens proteins described earlier in normal human aqueous humor

Key words alpha crystallin – aqueous humor – heterochromic cataract – lens induced uveitis – lens proteins – radioimmunoassay

Phacoanaphylactic uveitis and phacolytic glaucoma are believed to be secondary to pathological conditions in the lens It has been suggested that a humoral or cellular immune response to lens antigen is responsible but this remains to be proven (Burkey 1934 Maisel 1963 Halbert & Manski 1965)

With bovine lens extract as antigen a positive complement fixation test was found to be 70 times more frequent in sera from patients with uveitis than in sera from blood donors (Perkins & Wood 1964) Using a tanned cell hemagglutination technique Hackett & Thompson (1964) found anti lens antibodies in low titers in about half of the sera from healthy humans By a similar

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3rd, 6th and 9th day. Serum was collected on the 110th day by heart puncture.

Preparation of materials for immunoassay. The purified alpha crystallin was labelled with ^{125}I by the chloramine T method (Hunter & Greenwood 1962) in the following way. To ensure effective mixing, all solutions in the iodination procedure were added by blowing them forcibly from Carlsberg pipettes into the receptacle, taking care that there was no splashing and that no substance adhered to the pipette. To the original vial containing 2 μCi ^{125}I in 11 μl was added 25 μl 0.5 M phosphate buffer pH 7.4 (PB), 20 μl 0.1 M PB containing 10 μg alpha crystallin and 10 μl 0.1 M PB containing 25 μg chloramine T. Exactly 15 sec later 10 μl 0.1 M PB containing 50 μg meta bisulfite was added; 60 sec later 25 μl 0.1 M PB containing 50 μg NaI was added. Unbound iodine was removed by passage through a Sephadex G 25 superfine column with void volume 7 ml, height 15 cm and diameter 12 mm. As eluant 0.1 M PB containing 0.2 % bovine serum albumin and 0.1 % NaN_3 was used. The fractions corresponding to peak activity were pooled and diluted with phosphate buffered saline pH 7.5 (PBS) containing 0.2 % bovine serum albumin and 0.1 % NaN_3 until 5 μl of the solution gave 100 000 counts per 400 sec, and stored at -20 C until used.

Preparation of staphylococci. A stock solution of *Staphylococcus aureus* strain Cowan 1 was grown overnight on a liquid CCY medium (Arvidsson et al 1971), washed once with PBS, stirred with 0.5 % formaldehyde in PBS for 3 hours at room temperature, washed again with PBS and stored at 4 C as a 10 % suspension in PBS containing 0.1 % NaN_3. Before use the required amount was washed with and resuspended in 1 % Tween in PBS making a 2 % suspension.

Human material. Individual samples of aqueous humor were obtained in connection with cataract extractions or enucleations by puncturing the eye at the limbus before it was otherwise manipulated.

In all 53 samples were obtained.

Diagnosis	*Number of cases*
Senile cataract, mainly cortical	29
Senile cataract, mainly nuclear	4
Senile cataract, hypermature	8
Senile cataract, intumescent	2
Complicated cataract, chronic uveitis	2
Complicated cataract, heterochromic	2
Complicated cataract, absolute glaucoma	3
Clear lens (malignant choroidal melanoma)	3

technique Witmer (1957) found three cases of uveitis with fast developing cataractous changes in the lens to have high titer antilens antibodies in the aqueous humor

To evoke an immunological response lens antigens have to enter the aqueous humor Such lens antigens have been demonstrated under normal conditions in the aqueous humor of chickens and cows (Maisel 1962 Rao et al 1955) One lens antigen possibly gamma crystallin was demonstrated in the aqueous humor from near term human fetuses (Maisel 1963) Human aqueous humor obtained postmortem produced several precipitation lines when tested with anti lens antisera (Little & Langman 1964 Little et al 1965) These investigations were qualitative and seem to have been performed on pooled aqueous humor

It is the purpose of this paper to describe a method for quantitation of alpha crystallin in individual samples of aqueous humor

Materials and Methods

Preparation of antigen Alpha crystallin was isolated from clear sheep lenses and human cataracts The sheep lenses were removed immediately after slaughter Senile cataracts were collected from surgical extractions The material was immediately frozen and stored at –20°C until used Lens crystallins were isolated by zinc glycinate precipitation (Strittmatter & Ball 1955 Spector 1964) By electrophoresis in 1 % agar gel in 0 05 M barbiturate buffer pH 8 6 with field strength 5 V/cm for 2 hours the fractions showed the typical mobility of alpha beta and gamma crystallins respectively The sheep alpha crystallin fraction was estimated to be more than 90 % pure The contaminants moved as gamma crystallin in electrophoresis and were removed by passage through a Sephadex G 200 column The purified proteins were frozen in small aliquots and kept at –20 C

Preparation of antisera The semipurified alpha crystallin suspended in 0 05 M KCl 0 005 M Tris pH 7 3 and whole lens homogenate suspended in distilled water were used for immunization of rabbits Three rabbits were immunized with sheep alpha crystallin one with sheep lens homogenate three with cataract alpha crystallin and two with cataract homogenate Injections were given intramuscularly at two separate sites 10 mg (Lowry et al 1951) being used for each immunization The first dose was given in an equal volume of complete Freund s adjuvant Later injections were given without adjuvant on the 30th

37th 67th and 97th day Serum was collected on the 110th day by heart puncture

Preparation of materials for immunoassay The purified alpha crystallin was labelled with ^{125}I by the chloramine T method (Hunter & Greenwood 1962) in the following way To ensure effective mixing all solutions in the iodination procedure were added by blowing them forcibly from Carlsberg pipettes into the receptacle taking care that there was no splashing and that no substance adhered to the pipette To the original vial containing 2 μCi ^{125}I in 11 μl was added 75 μl 0 5 M phosphate buffer pH 7 4 (PB) 20 μl 0 1 M PB containing 10 μg alpha crystallin and 10 μl 0 1 M PB containing 25 μg chloramine T Exactly 15 sec later 10 μl 0 1 M PB containing 50 μg meta bisulfite was added 60 sec later 25 μl 0 1 M PB containing 50 μg NaI was added Unbound iodine was removed by passage through a Sephadex G 25 superfine column with void volume 7 ml height 15 cm and diameter 12 mm As eluant 0 1 M PB containing 0 2 % bovine serum albumin and 0 1 % NaN_3 was used The fractions corresponding to peak activity were pooled and diluted with phosphate buffered saline pH 7 5 (PBS) containing 0 2 % bovine serum albumin and 0 1 % NaN_3 until 5 μl of the solution gave 100 000 counts per 400 sec and stored at -20 C until used

Preparation of staphylococci A stock solution of *Staphylococcus aureus* strain Cowan I was grown overnight on a liquid CCY medium (Arvidsson et al 1971) washed once with PBS stirred with 0 5 % formaldehyde in PBS for 3 hours at room temperature washed again with PBS and stored at 4 C as a 10 % suspension in PBS containing 0 1 % NaN_3 Before use the required amount was washed with and resuspended in 1 % Tween in PBS making a 7 % suspension

Human material Individual samples of aqueous humor were obtained in connection with cataract extractions or enucleations by puncturing the eye at the limbus before it was otherwise manipulated

In all 53 samples were obtained

Diagnosis	*Number of cases*
Senile cataract mainly cortical	29
Senile cataract mainly nuclear	4
Senile cataract hypermature	8
Senile cataract intumescent	2
Complicated cataract chronic uveitis	2
Complicated cataract heterochromic	2
Complicated cataract absolute glaucoma	3
Clear lens (malignant choroidal melanoma)	3

Buffer 0 1 M PBS containing 0 2 % bovine serum albumin and 0 1 % NaN_3 was used for all dilutions in the following experiments

Experiments and Results

Pilot experiments showed that alpha crystallin could be detected by single radial diffusion in concentrations down to 100 μg/ml (Sandberg 1972a) Based on the opposite electrophoretic mobilities of alpha crystallin and gamma globulin counter electro immuno diffusion was conducted in order to increase the sensitivity (Sandberg 1972b) In this system alpha crystallin could be demonstrated in concentrations down to 10 μg/ml As this sensitivity was found to be insufficient to assay the alpha crystallin content in aqueous humor radio immunoassay was tried

The basic principle of radioimmunoassay In solution cold and labelled antigen will compete for antibody combining sites and at equilibrium the amount of labelled antigen bound to antibody will decrease as the concentration of cold antigen increases thereby defining a standard curve Different methods are used to separate free and bound antigens in immunoassay The following procedure (Folling & Kronvall 1973) is useful for antigens varying greatly in physico chemical properties and thus appeared useful for the present purpose This procedure is based on the capacity of protein A present on the surface of several strains of staphylococci to bind antibodies (Kronvall 1971) When staphylococci are added to the immunoassay mixture antibodies of the IgG class will bind to the staphylococci by the Fc fragment As a result antigen bound to IgG antibodies will sediment with the staphylococci by centrifugation whereas free antigen remains in the supernatant The amount of cold antigen present may then be determined by measuring the radioactivity of the sediment (Fig 1)

Antigen purity A pure antigen is a prerequisite for radioimmunoassay using labelled antigen By immunoelectrophoresis the sheep alpha crystallin solution obtained by zinc glycinate precipitation was found to contain at least two different antigens due to contamination with gamma crystallin As cataract alpha crystallin inhibited only one of the two precipitin lines between this alpha crystallin preparation and anti sheep alpha crystallin serum further purification of the antigen was needed before it could be used to assay human alpha crystallin After separation on a Sephadex G 200 column the peak eluted immediately after the void volume gave a single line in immunoelectrophoresis against a polyvalent anti lens antiserum By agar gel electrophoresis it gave

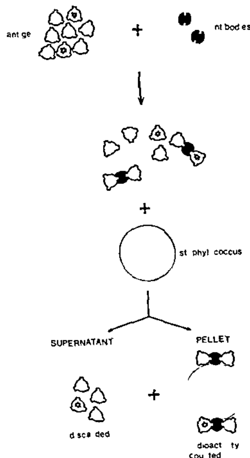


Fig 1
Schematic representation of principle for radioimmunoassay

a single band with the typical mobility of alpha crystallin Between the purified alpha crystallin and anti sheep alpha crystallin serum only one precipitin line was formed and this line was completely inhibited by addition of cataract alpha crystallin to the serum well

Evaluation of antisera Rabbit antisera against alpha crystallin from human cataracts and clear sheep lenses were examined for their ability to bind the highly purified labelled sheep alpha crystallin To 100 μl antiserum in dilutions from 1 100 to 1 1 000 000 was added 100 μl labelled alpha crystallin giving

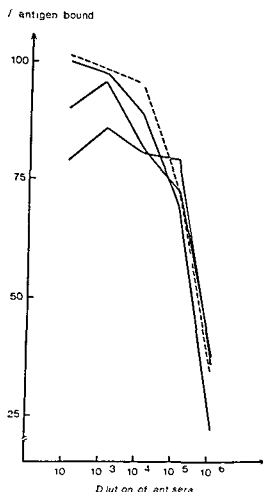


Fig 2
Evaluation of antisera With the two best antisera 100% of added labelled antigen was bound in dilution 1 100 The interrupted line indicates the serum used

approximately 25 000 counts per 200 sec Ten μl buffer were added and the mixture was incubated at 4°C for 19 hours Two ml 2% suspension of staphylococci were subsequently added followed by centrifugation for 20 min at 2 500 r p m The supernatant was discarded and radioactivity counted in the pellet Fig 2 shows that all antisera had high binding capacity for sheep lens alpha crystallin In the following experiments an antiserum dilution 1 500 000 was used

Standard curves 10 μl alpha crystallin in buffer 100 μl labelled alpha crystallin and 100 μl antiserum were incubated A standard curve was obtained covering the range from 0 02 to 10 μg alpha crystallin ml Attempts were then made to further increase the sensitivity No increase was obtained by diluting

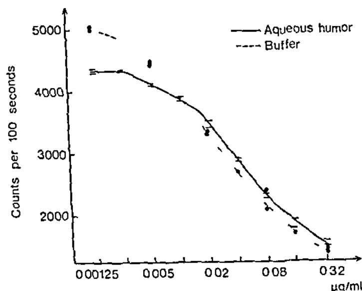


Fig 3

Standard curves for radio mmunoassay with buffer and aqueous humor as diluting agent respectively cf text

the antiserum and reducing the concentration of labelled antigen By preincubating cold antigen and antiserum before adding labelled antigen the sensitivity was increased The best results were obtained by 8 hours preincubation and 16 hours incubation which permitted detection of 0 00005 µg i e 0 005 µg/ml in a 10 µl sample (Fig 3)

Fig 3 shows two standard curves one in buffer and one in sheep aqueous humor In the right part of the diagram the curves run parallel and close to each other showing that alpha crystallin when present in aqueous humor is measured almost accurately when read on a buffer standard curve The left flat part of the aqueous humor curve shows that the sheep aqueous humor used as diluent contains 0 006 µg alpha crystallin/ml

Quantitation of alpha crystallin in human aqueous humor Fig 4 shows the distribution of the alpha crystallin content in the 53 examined samples In 46 samples the concentration was below the sensitivity of the test Five samples contained from 0 005 to 0 006 µg/ml and two samples contained 0 011 and 0 017 µg ml respectively These two samples were taken from the eyes with heterochromic cataract

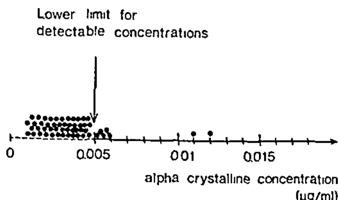


Fig 4
Alpha crystallin content in 10 µl samples of aqueous humor from 53 human eyes The two dots to the right represent the two samples from patients with heterochromic cataract

Discussion

Sheep alpha crystallin was chosen as the test antigen The organ specificity of lens antigens has long been recognized (Uhlenhuth 1903) It has been suggested that they express the evolutionary history of the lens (Manski et al 1960) Regarding the lens proteins the alpha crystallin displays the greatest organ specificity (Maisel 1964) One lens antigen specific for primates has been described (Maisel & Goodman 1965 Little et al 1965) Alpha crystallin from different mammalian species has been found to have similar immunological characteristics although different physical and chemical properties (Bjork 1968)

Alpha crystallin is easy to obtain in sufficient quantities During the evolution of a cataract the lighter crystallins gradually disappear until the cataract mainly consists of the albuminoid fraction and alpha crystallin (Maisel & Goodman 1965 François et al 1965 Spector et al 1971) It has also been suggested that the alpha crystallin is the essential antigen in the auto immune disease enophthalmitis phacoanaphylactica (Maisel 1963)

As the alpha crystallin concentration is usually below 0 006 µg ml in human aqueous humor one may assume that it has not been among those lens proteins earlier described in normal human aqueous (Little & Langman 1964 Little et al 1965) In those tests antisera were used against whole lens homogenate the aqueous humor was taken postmortem and the eyes enucleated before the samples were obtained

The sensitivity of the method presented 0 005 µg/ml could possibly be further increased by using anti human alpha crystallin serum rather than anti

sheep alpha crystallin As earlier mentioned it is assumed that the sheep lens does not contain antigenic determinants which are absent in human lenses when rabbit antisera are used The possible advantage of using an antiserum against human lens protein in the test is under investigation at present

The alpha crystallin used in the test was highly purified It seems safe to assume that no gamma crystallin will contaminate the solution after passage through the Sephadex G 200 column No beta crystallin was visible on gel electrophoresis agar gel diffusion gave no precipitin line between the alpha crystallin solution and anti beta serum and by immunoelectrophoresis only one line was formed betwen the alpha crystallin solution and a polyvalent anti lens serum

Among 53 samples of human aqueous humor 51 showed an alpha crystallin concentration of 0 005 to 0 006 µg/ml or below 0 005 µg/ml and normal sheep aqueous humor showed 0 006 µg/ml As the normal concentration is below or slightly above the limit for detection even a small pathological increase may be detected Two samples showed higher concentrations (Fig 4) and both were from eyes with heterochromic cataract Alpha crystallin has been demonstrated in normal human iris (Maisel 1963 Little & Langman 1964) One might there fore suspect that any aqueous humor with flare would contain increased amounts of alpha crystallin Five eyes with complicated cataract three with absolute glaucoma and two with uveitis had distinct aqueous flare In one of the latter the alpha crystallin concentration was 0 006 µg/ml in the other four it was below 0 005 µg/ml The aqueous flare in the heterochromic eyes was not as pronounced as in those referred to above

The demonstration of increased amounts of alpha crystallin in the aqueous humor from patients with heterochromic cataract suggests that the lens in this condition is somehow differently affected than in senile cataracts and the described complicated cataracts It is possible that the heterochromic uveitis causes a derangement of the lens capsule which permits leakage of lens proteins into the aqueous humor On the other hand increased amounts of gamma glo bulins have been described in aqueous humor from heterochromic eyes (Fran çois & Rabaey 1960) It may therefore be possible that the uveitis is an immune response to a primary release of crystallins from the lens

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The following review replaces the incomprehensible review in this periodical vol 51 p 629

Adnan H Halasa The Basic Aspects of the Glaucomas Charles C Thomas Sprin field Ill 1972 226 pages 68 figures Price $ 14 50

The book is intended for residents in ophthalmology and practising ophthalmolo ists It contains 4 sections namely a short historical review and classification of glaucomas a section on tonometry and tonography Section 3 deals with the primary glaucomas and the last section with the secondary glaucomas The latest years aspects of studies in the glaucoma disease such as cup/disc ratio Armaly s selective perimetry steroid glaucoma and thoughts about the mechanism of pupillary block are discussed The book does not include a description of the various surgical procedures in glaucoma with the exception of trabeculotomy in the treatment of congenital glaucoma Relevant ref erences and index The text is easy to read The book is rather short and cannot replace the classical textbook on glaucoma

S E Lorent en

Heilmann Klaus Augendruch Blutdruch und Glaukomschaden Ferdinand Enke Verlag Stuttgart 1972 92 pages 72 figures 4 tables Price DM 26

This book is installment No 61 of *Bucherei des Augenar tes* The contents are partly the results of precise scientific investigations and partly an assessment of the signi ficance of visual field examination in routine work with the glaucoma patient

In these few pages the author gives a concise well illustrated presentation of his subject consisting of the following chapters The significance of visual field examina tion for diagnosis prognosis and therapy

Increased intraocular pressure decreased blood pressure and visual field damage

Own investigations and methods

Intraocular pressure systemic blood pressure and visual field damage

An excellent little book on glaucoma in which the assessment of current glaucoma problems is relevant for all eye specialists both in hospitals and in private practice

F Kruse Hansen

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SPONTANEOUS REGRESSION OF A MALIGNANT MELANOMA OF THE CHOROID

BY

O A JENSEN and S RY ANDERSEN

A (year old man had for 1? years a lesion in the right eye clinically atypical of a melanoma The lesion and function of the eye was un hanged during the l years Since a melanoma seemed clinically most probable the eyeball was enucleated Histopathological examination re ealed a choroidal lesion with incipient invasion of the sclera abundant fibr s s calcifications and scattered neoplastic melanocytes confirmed by electron microscopy There was no necrosis and no inflammatory react n The d agnosis and the cause of regression are discussed and it is c n luded that this case represents a true spontaneous regression of a p imary malignant melanoma of the choroid

K y w d spontaneou regression of cancer – spontaneous regression of mal gnant mela mas sp n aneous regression of malignant choroidal melan ma

Read n a mod l ed form (by OAJ) at the 1 th Annual Meeting of the European Ophth lmic Pathology Society Dublin May 19 3

Spontaneous regression of malignant neoplasms has been reported a number of times. A large number of these cases are unacceptable because of inaccurate or controversial histological diagnosis. In a comprehensive survey Everson & Cole (1966) accepted 176 cases since 1900 as true regressions. Among these hypernephroma, neuroblastoma, choriocarcinoma and melanoma accounted for more than 50 %. Cases of malignant melanomas spontaneously regressed accounted for 11 %. These cases included two skin melanomas published by a Dane (Petersen et al. 1962) in British literature. In literature published in Denmark Olsen (1966) reported two cases and Brincker & Andersen (1972) two cases of spontaneous regression of malignant melanomas of the skin, and one of the present authors (Jensen 1963) reported a case of regression of metastases from a malignant choroidal melanoma. In the Everson & Cole series two cases are reported of spontaneous regression of presumed metastases from a malignant melanoma of the choroid. Reese et al. (1970) published two cases of presumed spontaneous regression of malignant choroidal melanomas without microscopic evidence. The case reported below we consider to be a true spontaneous regression in a primary malignant choroidal melanoma of long standing.

Material and Methods

Clinical history (Eye Dep. Kommunehospitalet, rec. no. 857/72)

A 65 year old man presented himself 12 years ago with a lesion in the upper temporal quadrant of the right eye. The vision of the eye was 6/9 + 3.00 sph., and a defect of the visual field was found in the lower nasal quadrant. No shadow on transillumination was observed. The diagnosis made by the ophthalmologist was in exudative retinitis.

Two years later (10 years ago) the patient suffered a head injury in a traffic accident but there were no successive ocular symptoms and no ophthalmoscopic examination was carried out. One month prior to enucleation he had pain in the right eye. Examination by the same ophthalmologist as 12 years earlier showed a completely unchanged lesion, visual acuity and visual fields. He was treated with steroids locally and scopolamine. In the interval he had not been exposed to X-rays, chemotherapy or hormonal therapy.

Examination in the Eye Department, Kommunehospitalet and in the tumour clinic of the Eye Pathology Institute revealed a greyish brown, round lesion in the upper temporal quadrant. It appeared to be solid, and on transillumination with cold fibre optic light a shadow was found corresponding to the lesion. An old detachment was seen at the sides. In the whole retina numerous atrophies and proliferations of pigment epithelium were noted but no serous detachment in the lower quadrants (Fig. 1). The intraocular pressure was subnormal in the right eye (10-16 mm). No inflammatory signs in the anterior chamber were observed. Although a diagnosis of a malignant

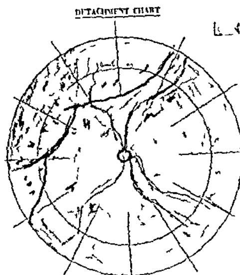


Fig 1
Detachment chart showing the greyish brown lesion in the upper temporal quadrant and serous detachment at the sides

ch ro dal melanoma was the most probable we found the lesion atypical and could n t exclude an exudative retinitis an epithelioma (adenoma) from the retinal pigment epithel um or an old haemorrhage

Pathology (Lab no 630 72 EM 57c)

The eyeball was of normal size and surface It was opened immediately after enucleat n The lesion was measured

Small pieces (1 mm³) of tumour tissue were immediately immersed in 6.5% caco dylate buffered glutaraldehyde of pH 3 for ? hours and postfixed in 2% OsO_4 for 2 hours dehydrated by ethanol followed by propylene oxide and embedded in epon 81? Ultra th n sections were cut with glass knives on a Reichert ultramicrotome OM U? ta ned with uranyl acetate and lead citrate and exam ned in a Zeiss EM 9S 2 electron microscope

R utine paraffin technique was appl ed to the rest of the eyeball after fixation in 4 % buffered neutral formaldehyde with 5% sucrose Deparaffinized hydrated sect ons were stained in the following ways

1) Haematoxylin eosin,) haematoxylin phloxine safranin 3) alizarin red S 4) the on Kó sa method 5) murexid staining 6) PAS 7) alcian blue 8 GX, 8) Masson Fon tana sta n ng 9) iron sta n ng

Results

The lesion was flat, about 12 x 10 x 2 mm and black on the cut surface

Light microscopical examination showed in the posterior choroid a flat intumescence (Fig 2) with growth into the sclera and probably along the vessels (Fig 3) Large areas with fibrosis were seen and calcifications identified by the alizarin red S von Kossa and murexid stainings were found mainly in the inner parts of the lesion (Fig 2) Large polymorphous cells were scattered between strands of connective tissue some spindle shaped some multinucleated and some with a foamy cytoplasm (Fig 4) These were PAS and alcian ne

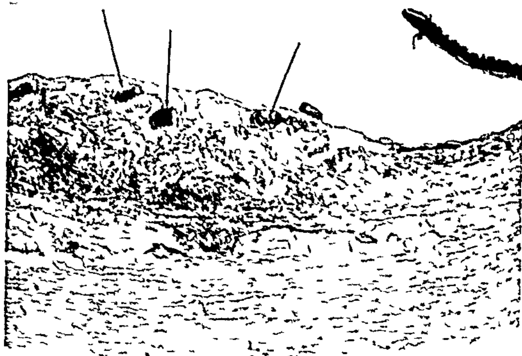


Fig 2

Survey of the choroidal lesion with darker areas and lighter connective tissue parts as well as calcifications (arrows) Haematoxylin phloxine safranin (x 25)

Fig 3

Invasive growth into the sclera The normal scleral boundary is at the arrows A thick walled vessel is seen in the intrascleral part (double arrow) Haematoxylin phloxine safranin (x 100)

Fig 4

Several cells with a foamy cytoplasm (arrows) between connective tissue strands Haematoxylin eosin (x _ 5)

Fig 3

Fig 4

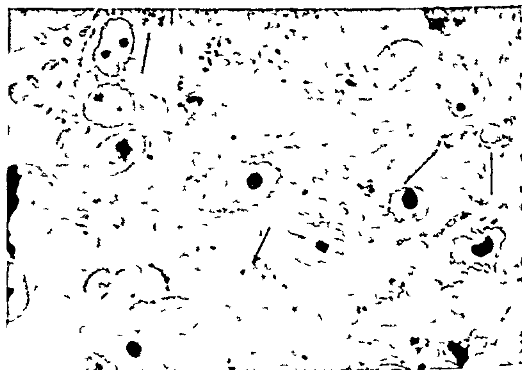


Fig 5
Epon embedded 1μ section showing melanocytic cells with intracytoplasmic melanin granules (arrows) Safranin O (× 1000)

ative. Mitoses were not observed Some melanophages were noted but several cells had discrete intracytoplasmic granules These were black with the Masson Fontana stain like melanin granules Iron staining was negative In our opinion they were undoubtedly neoplastic melanocytes (Fig 5) Vessels in the lesion were scarce but some were thick walled though no occluded vessels (Fig 3) were found The vessels outside the lesion were normal No unusual inflammatory signs were observed and particularly there were no large areas with necrosis and heavy inflammatory infiltration The pigment epithelium was intact

Electron microscopy showed several widespread cells with melanosomes in various stages of melanization Some of these had large irregular nuclei with large nucleoli but some had more regular round nuclei The plasma membrane was usually regular and the intercellular spaces broad (Fig 6) These cells mostly correspond to malignant melanoma cells of the mixed cell type (Fgeberg & Jensen 1972) Cells with distended mitochondria defective plasma membrane and broad perinuclear cisterns were seen but these were lying in areas with poor fixation and therefore cannot be regarded as cells with signs of degeneration

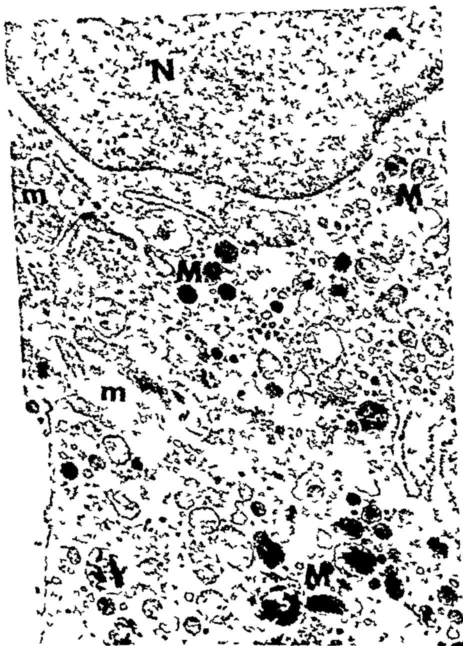


Fig 6

El t n mi graph of tum ur cell with melanosomes in various phases of development N nu leus M melan s mes m m tochondria (20000)

Discussion

A number of questions arise concerning the clinical and histopathological facts in this case First is it in fact a melanoma? The lesion had choroidal localization and contained large polymorphous cells with the light microscopical and all ultrastructural features of melanocytes These melanocytes had no similarity to normal choroidal melanocytes but had characteristics similar to malignant melanoma cells described by Egeberg & Jensen (1972) Most cells belonged to the mixed group Although melanophages were seen several cells without complex melanosomes (as seen in melanophages) were observed Haemosiderophages were not observed making an old vascular lesion unlikely

We interpret the large cells with a foamy cytoplasm as balloon cells sometimes seen in nevi and melanomas They may have contained glycogen or fat removed during the preparation

No retinal changes above or in addition to the lesion were found excluding the clinically suspected exudative retinitis The fact that the lesion had invaded the sclera adds further evidence to our conclusion that it is a malignant melanoma The present tumour is unique in having large areas of fibrosis and calcification To the best of our knowledge the latter has not previously been described in malignant choroidal melanomas

The next question to be answered is whether the melanoma arose 12 years ago or whether it arose later in an old pathological change The fact that malignant melanomas in eyes with previous lesions of several kinds are always solid and of common morphology makes the latter assumption unlikely Tumours in eyes with previous lesions of the morphology here presented have neither been described in the literature nor are found in our collection

Another unique feature of this case is the absence of inflammatory reaction Necrosis is very often seen in malignant uveal melanomas as a result of the tumour outgrowing its vascular supply This necrosis may be of such an extent that identification of tumour cells becomes impossible In contrast to the present case the tumour in these instances is usually large and the eyeball heavily inflamed and affected by the toxicity of the necrotic tissue Intractable glaucoma is usually the result and extraocular extension of viable tumour tissue is often observed The history in these cases is usually rather short seldom more than 2 years Reese (1963) stated that pigmented tumours in general may show spontaneous necrosis and regression which cannot be explained by insufficient blood supply The cause is unknown in these cases The same process may unquestionably take place in uveal melanomas I have seen it in an iris melanoma and I am sure it can take place in melanomas elsewhere in the uvea (Reese 1963)

Later Reese et al (1970) gave a survey of cases with necrosis and inflammation and considered these changes to be an immunological response causing tumour regression Mishima (1967) suggested that the tendency of spontaneous regression of melanoma in some cases can result from high activity of acid hydrolases in these neoplasms and a high autoproteolytic activity of transplantable melanomas of the Syrian golden hamster was demonstrated by Zbytniewski & Drewa (1972) In the present case no vascular anomalies were found in posterior ciliary choroidal or retinal vessels The thick walled vessels in the lesion (Fig 3) are usually seen in every type of malignant neoplasm We therefore conclude that a vascular genesis is unlikely

Since immunological phenomena in tumour regression are well known and have even been used in the therapy of cutaneous malignant melanomas (Krementz 1970) and since autoimmune reactions in uveal melanomas have been found (Rahi 1971) we may possibly ascribe the regression in our case to immunological factors although inflammatory reaction and lymphocyte infiltration were absent In any event the history of twelve years with an unchanged function of the eye and unchanged size of the lesion the extensive fibrosis and the calcifications together with the evidence of perivascular invasion of the sclera and demonstration of undoubtedly neoplastic melanocytes lead us to the conclusion that this case is a true spontaneous regression of a malignant choroidal melanoma whatever the cause may be

Acknowledgment

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RETINOBLASTOMA WITH NECROSIS OF CENTRAL RETINAL ARTERY AND VEIN AND PARTIAL SPONTANEOUS REGRESSION

BY

S RY ANDERSEN and O A JENSEN

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Read in a modified form (by S R A) at the 11th Annual Meeting of the European Ophthalmic Pathology Society Helsinki *June 19 ?*

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RETINOBLASTOMA WITH NECROSIS OF CENTRAL RETINAL ARTERY AND VEIN AND PARTIAL SPONTANEOUS REGRESSION

BY

S RY ANDERSEN and O A JENSEN

A case of partial spontaneous regression of retinoblastoma in one eye and fatal retinoblastoma in the other eye is described In the spontaneously regressed retinoblastoma necrosis of the central retinal artery in the lami na cribrosa and necrosis of the central retinal artery and vein in the disc were demonstrated No DNA deposits were established in the haematoxy ph l c retinal vessels some of which were thrombosed The most likely explanation of the spontaneous regression is that the tumour had grown out of its vascular supply with secondary necrosis of the tumour and haem orrhage on the disc followed by necrosis of the central retinal vessels An inflammatory reaction in the necrotic tumour tissue might be the cause of the simultaneous rhin tis and pleuritis An immunological reaction in the tumour or a primary inflammation outside the eye causing the pan ophthalmitis have also been considered

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Spontaneous regression of retinoblastoma occurs infrequently In 1969 Boniuk & Girard reported about 40 cases from the literature In most of the early cases the diagnosis was made solely on the basis of clinical findings In 1962 Boniuk & Zimmerman made a histological study of 14 enucleated eyes that contained a regressed retinoblastoma In six of these cases a unilateral retinoblastoma had undergone spontaneous regression In eight cases the disease was bilateral with spontaneous regression in one eye and a viable tumour in the other eye The stage of regression seemed to vary from case to case If the eye had been enucleated shortly after it had become phthisical the calcified tumour cell areas were usually surrounded by necrotic tumour cells If enucleation had been delayed after the onset of phthisis bulbi fibrous connective tissue or osseous tissue often separated or surrounded the faint remains of neoplastic cells Not all eyes with regressed retinoblastoma were phthisical and not all retinoblastoma tissue in phthisical eyes was completely regressed In none of the histopathologically investigated eyes was serial sectioning carried out and accordingly the central retinal vessels were not identified in any of the cases

In an eye with partial regression of retinoblastoma following irradiation Lee (1973) has demonstrated a thrombus in the central retinal artery

Mullaney (1969) suggested the likelihood of circulating anti DNA antibodies in retinoblastoma patients and a possible antigenic role of retinoblastoma cells particularly in the case of spontaneous regression

We have had occasion to investigate one case of spontaneous regression of retinoblastoma with necrosis of the central retinal vessels

Material and Methods

Clinical history

A boy without a family history of eye disease the second of two children was delivered normally From birth the mother had noticed a slightly divergent right eye which looked more translucent when viewed sideways No other disease during early childhood

On December 13th 1964 at the age of 15 months his temperature suddenly rose to 39° C Two days earlier the mother had noticed an enlarged pupil a brown iris and swelling of the lower conjunctiva of the right eye

On admission to the Department of Ophthalmology Gentofte Hospital (Rec no I 315 – 64/65) a right sided pleuritis was diagnosed The right eye was inflamed immovable and protruding The cornea was greyish and lustreless the anterior chamber contained blood and the pupil was dilated but tension was normal An ethmoiditis with panophthalmitis thrombosis of the cavernous sinus or possibly a tumour of the right eye was considered and penicillin treatment was ordered X rays however revealed no

signs of ethmoiditis On the next day fibrin and a 1 mm hypopyon were noticed in the anterior chamber The conjunctival pus contained staphylococci

Ophthalmoscopial examination of the right eye was impossible but was normal in the left eye

During the following days the temperature slowly fell to normal and the sedimentation rate fell from ?0 to 7 mm 1 hour A purulent rhinitis disappeared and a massive net like shadow in the right lung vanished A battery of blood tests was normal Vanillin mandelic acid and noradrenaline excretion in the urine were normal The otologists still felt that the child had most probably had an ethmoiditis with orbital phlegmon

Four weeks after admission the right eye began to become phthisical and it was st ll impossible to view the interior part In the left eye two small white retinal tumours were now observed in the upper temporal quadrant and the diagnosis of bilateral retinoblastoma was made

On January 19 1965 5 weeks after admission the soft phthisical *right eye* was enucleated without any treatment After the enucleation prophylactic telecobalt radiation was given to the right orbital socket (4,350 rad/18 days) at the Radium Centre Århus The two tumours in the left retina were radiated by Rosengren Tengroth s cobalt ball (?4 hours each tumour) tumour dose in 1 mm scleral d pth 10 ?00 rad) at the Department of Ophthalmology University of Århus (Rec no 86?4 65)

On May 19 the child was re admitted to hospital because of vomiting convulsive attacks and loss of consciousness No recurrence was found in the right orbital socket or in the left eye The child died on May ?? 19? 21 months old 5 months after his fir t febrile attack

Autop y at Glostrup Hospital (Rec no 353/65) showed diffuse invasion of undifferentiated retinoblastoma cells in the subarachnoid space of the brain cerebellum and medulla oblongata but no tumour tissue in the right orbital socket or *in the internal* rgans Bronchopneumonia of the right lung oedema of both lun?s and a localized acute *periton tis* secondary t rupture of the pylorus (caused by a stomach pump) were n ticed

Ocular Pathology

Macroscopical examination

Right eye (Eye Path Inst no 26/65 a) Enucleated 5 weeks after onset of eye symptoms A phthisical eye measuring 18 x 1? x 21 mm The interior was filled with a greyish and bloody mass (Fig 1) Decalcification in formic acid and sodium formiate (Kristensen s fluid) for 4 days

Left eye (Eye Path Inst no 2? 5? ? a + b) Removed at autopsy The 22 x 21 ? mm eye was obliquely divided to reveal a peripheral scar in the upper temporal quadrant

Right orbital tissue from the socket (Eye Path Inst no ?66 65 Removed at autopsy



Fig 1

Phthisical right eye filled with a greyish and bloody mass (Eye Path Inst no 26 65 a)

Fig 2

Interior of right eye with a partly necrotic inflamed mass with haemorrhages and almost completely calcified retinoblastoma cells (× 40)

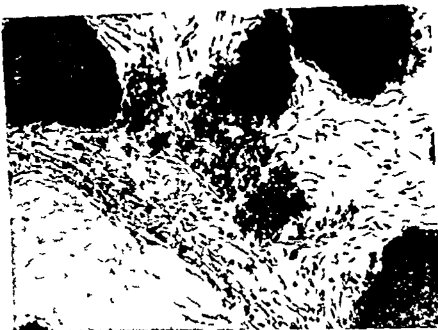


Fig 3

Partly calcified retinoblastoma cells in central retinal mass no mitoses or rosettes (105)

Staining Deparaffinized sections were stained by 1) haematoxylin eosin and 2) van Gieson

In addition sections from the right eye were stained by 1) haematoxylin phloxine safranin 2) PAS 3) alcian blue (Eskelund's modification) 4) Gram 5) Brown Brenn 6) Grocott 7) toluidine blue 8) reticulum stain 9) iron stain 10) Unna Pappenheim 11) gallocyanin chromalum (Einarson) 12) Feulgen 13) alizarin red S 14) von Kóssa and 15) acid iron orcein

Microscopical examination

Right eye 240 serial sections in the area of the disc and sections at random outside the disc area showed a phthisical eye The interior was filled by a partly necrotic inflamed scarred mass with haemorrhages and a massive proliferation of ciliary and retinal pigment epithelium The central mass contained almost completely calcified islands of retinoblastoma cells without mitoses or rosettes (Figs 2 & 3) In some of the sections dysplastic degenerated retinal tissue was seen In the posterior choroid the optic nerve and behind the sclera islands of



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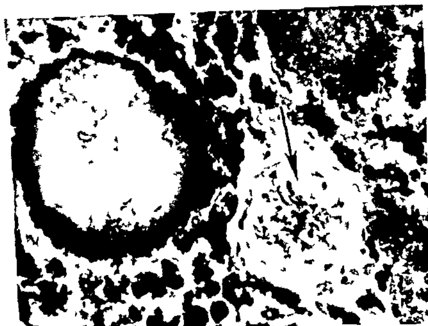


Fig 5
Central mass with calcifications and a thrombosed vessel (arrow) probably an arteriole surrounded by melanophages (× ?80)

interfering effect due to the calcification or to the exposure by Kristensen's decalcification fluid (formic acid and sodium formate) which had been used on our specimen In the control specimens the DNA either free or in blood vessels could still be demonstrated even after immersion of the sections in the decalcifying fluid for up to 3 weeks

The choroidal arterioles showed remarkable hyperplastic sclerosis perhaps more than the moderate inflammation could be assumed to cause (Fig ?) Some of the posterior ciliary arteries outside this eye were also somewhat sclerosed (Fig 9) but the surrounding connective tissue was rather inflamed These arteries were enucleated with the eye and had not been treated by radiation either The Feulgen stain reaction was positive in the calcified central intraocular areas

The von Kóssa and alizarin red S stains for calcium were negative this was to be expected as the specimen had been decalcified Stains for bacteria and fungi were negative



Fig 4
Central retinal artery (arrows) in lamina cribrosa with necrosis (left half of picture)
Haematoxylin phloxine safranin (× 280)

somewhat better preserved retinoblastoma cells with some mitoses were seen The central retinal artery was normal in the optic nerve but suddenly became necrotic in the lamina cribrosa (Fig 4) The central retinal vein which was surrounded by some lymphocytes in the optic nerve also became necrotic in the optic nerve head The disc was covered by a large haemorrhage and in this necrotic area only the vein could be followed Its walls were necrotic but no thrombi were seen In the scarred mass some thrombi were seen in smaller vessels probably arterioles (Fig 5) These vessels were surrounded by melanophages Many of the smaller retinal vessels in the central scarred mass including the capillaries were deeply haematoxyphilic (Fig 6) and some of these were PAS positive Stains for DNA (gallocyanin Unna Pappenheim and Feulgen stains) were negative as far as the vessels were concerned

Mullaney Dublin found negative staining for DNA in the non nucleated deposits in some of our sections with Feulgen and the modified gallocyanin method for nucleic acids according to de Boer & Sarnaker (1956) In a series of tests on control retinoblastoma cases Mullaney excluded the possibility of an

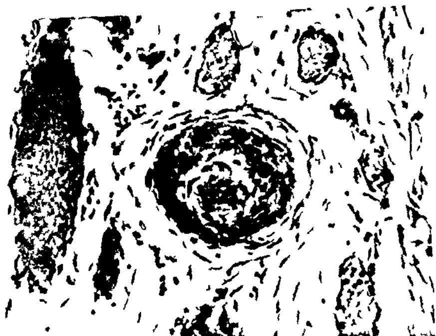


Fig 8

Hyperplastic arteriolo sclerosis in posterior ciliary arteriole behind the eye (× 280)

The right orbital tissue from the socket was scarred with endothelial prolifera tion and sclerosis of the vessels due most probably to radiation changes but without tumour cells

The left eye showed scarred tissue in the retina but no tumour recurrence in the retina was seen The optic nerve and sheaths were invaded by viable retinoblas toma cells with many mitoses The vessels except for the radiated areas had a n rmal appearance

Discussion

There is no doubt that the right eye of this child contained a retinoblastoma with spontaneous regression of the retinal and vitreal part of the tumour The tumour tissue in the choroid and outside the sclera was better preserved especi ally the extraocular part

The central retinal artery was necrotic in the lamina cribrosa The central retinal artery and vein were necrotic in the optic disc which was covered by a

Fig 6

Haematoxyphilic capillaries (arrows) in the central mass (× 105)

Fig 7

Choroidal arterioles with remarkable hyperplastic sclerosis The surrounding choroidal tissue is moderately inflamed (× 280)

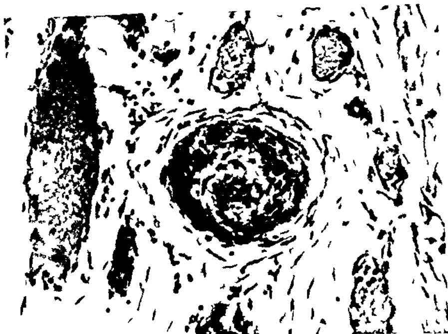


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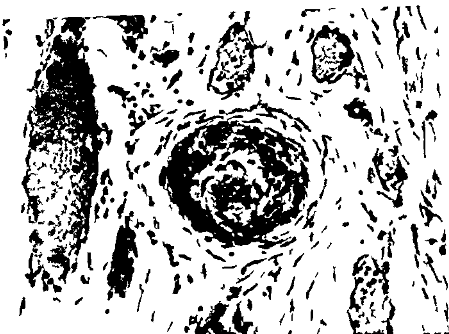


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The central retinal artery was necrotic in the lamina cribrosa The central retinal artery and vein were necrotic in the optic disc which was covered by a

large haemorrhage separating the disc from the scarred central retinal and vitreal mass

Several explanations are possible

1) The tumour might have grown out of its vascular supply This might have caused necrosis of the retinal and vitreal tumour followed by a haemorrhage on the disc consequently causing necrosis of the central retinal vessels and thrombosis of retinal arterioles The inflammatory reaction in the necrotic tumour and retina might possibly be secondary to the necrosis The inflammatory reaction might have spread to the choroid and caused the rhinitis and pleuritis This explanation seems to us the most probable

2) An immunological reaction in the tumour as a primary or coexisting factor must be considered The severe choroidal arteriolar sclerosis and the slighter one in the extraocular posterior ciliary arteries might suggest this but it cannot be disregarded that the sclerosis might be secondary to the inflammation

In the case of an immunological reaction it is difficult to understand why the central retinal vessels posteriorly in the right optic nerve and all the vessels in the left eye do not take part in the hypothetical immune reaction in the vessels The haematoxyphilic retinal vessels in the regressed tumour do not appear to contain DNA deposits and therefore an immune reaction in the retinoblastoma cells and in the vessels seems very unlikely

3) A primary inflammation outside the eye e g the rhinitis or the pleuritis being the cause of the panophthalmitis of the right eye appears to us to be less probable

The clinical history starting with inflammation of the right eye does not support this

The stainings illustrated in the microphotographs are haematoxylin eosin unless otherwise stated

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OCULAR HYPERTENSION AND GLAUCOMATOUS OPTIC NERVE DAMAGE

BY

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The incidence of glaucomatous optic nerve damage in ocular hypertension of varying degrees was studied. It rose from 7 % with an intraocular pressure of 25-29 mmHg to 70-80 % with an intraocular pressure of over 45 mmHg. The increase was sharpest when the pressure rose above 34 mmHg. The incidences were higher for eyes with pseudoexfoliation syndrome than for eyes without pseudoexfoliation. The susceptibility of the eyes to elevated intraocular pressure is distributed over a wide area. Perhaps only a minority of eyes develop optic nerve damage if the intraocular pressure keeps below 30 mmHg. There are possibly eyes whose ability to resist intraocular pressure is not overcome until the pressure is over 34 mmHg.

Key words: ocular hypertension – optic nerve – simple glaucoma – capsular glaucoma – pseudoexfoliation syndrome

Many mass surveys have shown that ocular hypertension above 22 mmHg is a more common disorder than glaucomatous optic nerve damage (OND) (Strömberg 1962; Hollows & Graham 1966; Norskov 1970 a, b; Armaly 1972). On the other hand, OND has often been found in eyes with an intraocular pressure below 22 mmHg. It is evident that the ability of individual eyes to resist intra-

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ocular pressure varies It seemed of some purpose to study the frequency of OND in ocular hypertension of varying degrees in order to obtain information about the dangerousness of different pressure levels

Material and Methods

The material consisted of patients who were found in 1969 1972 to have OND and/or elevated intraocular pressure above 24 mmHg and open chamber angles they were under observation in the Eye Department Regional Hospital of Lahti Secondary hypertensions were not included The series included four patients with OND whose hypertension had been diagnosed earlier but who had neglected the treatment prescribed

The routine was to admit all patients who had a finding indicative of OND and/or ocular hypertension repeatedly above 24 mmHg In the majority of cases the intraocular pressure was measured several times on an outpatient basis Examinations in the ward included visual acuity diurnal curve with Schiotz tonometer gonioscopy tonography (Mueller & Co) visual fields with Goldmann perimeter and after dilatation of the pupil a slit lamp examination and ophthalmoscopy The estimation of optic cupping was made with a few exceptions by the same person (P I) binocularly with a slit lamp (Hruby lens or contact lens)

The highest pressure recorded repeatedly was used in the study as the pressure value for an individual eye Effective therapy had been instituted immediately on some eyes with a high intraocular pressure and for those the initial pressure was used as the pressure value For patients with binocular OND or binocular hypertension without OND the value of the eye with the higher pressure was used

The following defects in the visual field were considered as glaucomatous if no other reason was known scotomata in the Bjerrum area peripheral and central nasal steps sector shaped scotomata and tubular narrowing Baring of the blind spot was not included (Drance 1972a)

The study was based on 307 patients Ninety six were considered to have OND in one or both eyes (132 eyes) Eighty six patients had both cupping of the disc and visual field loss Seven patients had cupping of the disc (mostly a definite asymmetry of the discs and tensions) without a demonstrable field defect Three patients had a field loss associated with a disc that was not considered cupped

Fifty three (55 %) of the OND patients and 143 (68 %) of the other hypertension patients were women. The mean age of the OND patients was 69.8 years and of the other hypertension patients 63.1 years. Pseudoexfoliation was seen in 134 eyes of 99 patients.

Results

The incidence of OND in the material increased slightly at first as the intraocular pressure rose (Table I). At the pressure reading 35 mmHg there was a steep climb in the incidence. The rising trend ceased above the 40 mmHg level when the incidence was 60–80 %. The number of patients with such a high intraocular pressure was so small that the percentages are not accurate.

The incidence of OND was higher at most tension levels for the eyes with pseudoexfoliation syndrome than for the eyes without (Table II). The difference is statistically significant ($P < 0.01$).

The intraocular pressure remained below 30 mmHg in 68 % of the OND eyes without pseudoexfoliation (glaucoma simplex). The eyes were more nume

Table I

Incidence of glaucomatous optic nerve damage (OND) in different grades of ocular hypertension

Intraocular pressure (mmHg)	Total no. of patients	No. of patients with OND	Incidence of OND
< 20	–	1	–
20–24	500*	14	3 %
25–29	133	9	7 %
30–34	64	9	14 %
35–39	27	14	52 %
40–44	23	14	61 %
45–49	11	8	73 %
50–54	18	15	83 %
55–59	6	5	83 %
60 <	10	[illegible]	0 %

* an estimation on the basis of Norskov's (1970b) study on ophthalmic practice

Table II

Incidence of glaucomatous optic nerve damage (OND) in different grades of ocular hypertension for eyes with pseudoexfoliation syndrome and for eyes without

Intraocular pressure (mmHg)	With pseudoexfoliation syndrome		Without pseudoexfoliation syndrome	
	Total no of eyes	No of eyes with OND	Total no of eyes	No of eyes with OND
< 20	–	2	–	1
20–24	–	1	–	24
25–29	26	4	229	13
30–34	27	5	71	8
35–39	20	12	29	6
40–44	21	12	10	7
45–49	6	6	5	2
50–54	18	15	2	2
55–59	6	5	1	0
60 <	7	6	3	1

rous in the lower than in the upper twenties The intraocular pressure was below 30 mmHg in only 10 % of OND eyes with pseudoexfoliation (glaucoma capsulare)

The incidence of pseudoexfoliation increased considerably when above 35 mmHg It was encountered in 20 % of the OND eyes with a pressure below this level The corresponding percentage was 73 when the pressure was above 35 mmHg

Comment

One difficulty about a study of intraocular pressure is denoting the pressure of an individual eye by a single figure In a study on glaucoma the best value is probably the repeated upper limit of the pressure variation because glaucoma conceivably develops when the tolerance of the eye is exceeded for some time at any rate The frequency distribution of intraocular pressures obtained in this way does not correspond to a distribution based on a single pressure recording The value employed in our study did not however differ much on the whole from the initial pressure The pressure reducing effect of hospitalisation

which has been established in many connections was obviously the reason why even the peaks of the pressure curve during the hospital stay often did not exceed the outpatient reading of the first examination

It should be remembered when evaluating hypertensive patients seeking medical advice that the material is selected ONDs and severe hypertensions are probably overrepresented

The incidences of OND in our series were astonishingly low One possibility for the low incidences could be failure to verify OND in some eyes The judgement of optic nerve cupping is not easy in borderline cases and it is difficult to get an infallible result in perimetry The results in perimetry and ophthalmoscopy however conformed well with each other The examinations of visual fields were made by personnel who were not informed of the optic nerve state If by chance they saw the annotation in the case report they had no knowledge of its significance One person made the ophthalmoscopies so that the results would be mutually comparable at the different levels of intraocular pressure Earlier studies have also established the high incidence of raised intraocular pressure compared with OND Especially Strömberg s (1962) findings support the present results

At the high pressure levels above 40 mmHg there were 19 patients out of 68 without observable OND A possible explanation is that the severe hypertension often provoked symptoms and brought the patient to the ophthalmologist at an early phase

The dangerousness of a pressure level could be expressed accurately only if the number of wrong negatives (eyes in which the optic nerve was not yet damaged) were known Many follow up studies give reason to suppose that the number of "wrong negatives is not very high among patients with an ocular hypertension below 30 mmHg (Linnér & Stromberg 1966 Armaly 1969 Norskov 1970c d Perkins 1973) At the high pressure levels above 40 mmHg perhaps all eyes will develop OND and then wrong negatives will account for half the incidence of diagnosed OND

The OND incidence at a certain pressure level includes those patients who would have developed OND already at a lower pressure If we deduct from the OND incidence of a pressure level that of the next lower level the result perhaps shows the number of patients whose ability to resist intraocular pressure has extended to the range of the higher pressure level

The incidence of OND increased sharply as the pressure rose above 34 mmHg and seemed to level off only after 40 mmHg Our results therefore suggest that the ability of most patients to resist intraocular pressure is perhaps exceeded at the pressure level 30 39 mmHg and only a minority of the patients with spontaneous pressure peaks below 30 mmHg develop OND Many eyes perhaps

tolerate spontaneous intraocular pressure increases up to the lower thirties One must bear in mind it is true that especially at the tension level 30–34 mmHg the great majority of eyes were at the lower limit of the category This has a lowering effect on the OND incidence

When the intraocular pressure was above 40 mmHg all the tension levels seemed to be equally dangerous This is the result if all eyes develop OND at these pressure values

The intraocular pressure is below 30 mmHg in most eyes with simple glaucoma This is because when the pressure decreases and the incidence of OND declines the number of eyes rises so sharply that the greatest number that exceed their pressure tolerance are to be found at the 20 29 mmHg level Low pressure tolerance seems to be a general feature of eyes with simple glaucoma together with mild hypertension

It is known that the presence of pseudoexfoliation syndrome in eyes with hypertension has impaired outflow and raised the intraocular pressure (Pohjanpelto 1973) In our series ocular hypertension was seldom severe without the pseudoexfoliation syndrome The incidence of pseudoexfoliation increased as the intraocular pressure rose It was found in most eyes with an intraocular pressure above 34 mmHg As a result of this and the increase in OND as the pressure rose eyes with glaucoma capsulare generally had a higher intraocular pressure than eyes with glaucoma simplex This is obviously the main reason why the prognosis is poorer in capsular than simple glaucoma (Tarkkanen 1962 Hørven 1966) and why the incidence of pseudoexfoliation is increased in severe forms of glaucoma (Aasved 1971) In addition our results suggest that the eyes with pseudoexfoliation are probably more liable to develop OND than those without pseudoexfoliation

The susceptibility of eyes to intraocular pressure elevation appears to be distributed over a wide range This agrees well with the observations stating that there are many important factors in the development of OND (Armaly 1970 Drance 1970b Hayreh 1972 Leighton & Tomlinson 1973)

Since one is not able to determine beforehand whether an individual eye can resist a given intraocular pressure the prevention of OND is a very difficult problem Even mild cases of ocular hypertension may be justified for treatment if the patient responds and no risk or inconvenience is involved Therapy has the advantage of giving an opportunity for regular observation but perhaps one should not cause the patient anxiety by using the term glaucoma If there is a spontaneous rise in pressure to above 30 mmHg the risk of OND is so great that medical therapy should perhaps be instituted despite the possible overtreatment of some patients The presence of pseudoexfoliation syndrome speaks for the early treatment

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CORNEAL AND FUNDUS CHANGES IN FAMILIAL LCAT DEFICIENCY

BY

I HØRVEN K. EGGE AND E GJONE

Two patients with Familial LCAT deficiency are presented Deposits of minute grayish dots in the cornea gave it a nebulous appearance. Near the limbus these dots increased in numbers and formed a grayish circular band resembling an arcus lipoides These bilateral changes have been apparent from early childhood The presence of arcus lipoides corneae before puberty should make the clinician think about this disease

Fundus changes including retinal hemorrhages disc protrusion and rupture of Bruch s membrane may be explained by deposits of pathological lipid material into these structures Nervous manifestations have been found in other dyslipoproteinemias but have not previously been described in Familial LCAT deficiency

Key words angioid streaks – corneal deposits – dyslipoproteinemia – Familial LCAT deficiency – papilledema – retinal hemorrhage – visual field loss

Familial LCAT deficiency (lecithin cholestorol acyltransferase deficiency) was first described in three adult Norwegian sisters as a new clinical syndrome in 19((The characteristic clinical features (Gjone & Norum 1968) were corneal opacities proteinuria normochrome anemia and a turbid/milky plasma Total

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cholesterol phospholipids and triglycerides were elevated in plasma of the two eldest sisters and the concentrations of cholesterol esters and lysolecithin were below normal in all Absence of plasma lecithin cholesterol acyltransferase (LCAT) was found as the fundamental defect (Norum & Gjone 1967) Erythrocytes had target cell appearance and contained increased amounts of cholesterol and lecithin Foam cells were found in bone marrow and kidney glomeruli

In all six Scandinavian patients described so far with Familial LCAT deficiency and the new case presented in this paper corneal opacities have been a characteristic feature In some patients it was noted for the first time before puberty A detailed description was given in 1969 (Gjone & Bergaust 1969)

Since the original description of the corneal opacities in Familial LCAT deficiency the oldest of the three Norwegian sisters has developed fundus changes with disc protrusion and reduced vision A new patient who will be fully described elsewhere had changes in the retinal vessels and in Bruch s membrane close to the optic disc Both the corneal and fundus changes are believed to be secondary to the lipid disorders and will be described in detail here

Case Reports

Case 1

The female patient A R born in 1933 was first diagnosed as having Familial LCAT deficiency in 1966 (Gjone & Norum 1968) Proteinuria was noted in 1957 and anemia in 1966 As described previously (Gjone & Bergaust 1969) she had marked corneal opacities Vision had been good until then and neither she nor any of the other patients with this disease had any ocular complaints

During the winter of 1970 her kidney function rapidly deteriorated and from April 1970 she was on hemodialysis until kidney transplantation was performed in January 1973

The ocular function was normal until summer 1971 Since then a gradual reduction has occurred in visual acuity and fields along with pathological changes in both optic discs

FINDINGS

1 *Corneal opacities* The corneal opacities were first seen by slit lamp examination in March 1967 although they had presumably been present from early childhood The opacities were localized in the parenchyma as innumerable minute grayish dots giving the cornea a nebulous appearance These dots were abundant and evenly distributed in all layers of the stroma without affecting the epithelium endothelium or Descemet s membrane Near to the limbal area the dots increased in numbers and formed a dense grayish circular band resembling an arcus lipoides senilis The arcus was separated

from limbus by a relatively clear zone of corneal tissue and the outer borderline of the arcus was somewhat irregular and blurred because of variation in the number of stromal dots These corneal changes were present in both eyes and to some extent progressed with time until the hemodialysis was commenced in 1970 No lid xanthelasmata were present

2 *Papillary manifestations* The fundi appeared normal until May 1971 when both discs showed blurred borders with several flame shaped hemorrhages at and near the disc margins as shown in Fig 1a No exudates and no A V phenomena were found and the retinal artery caliber appeared normal The blood pressure was 140/90 mmHg (The blood pressure had been within normal limits from June 1966 until the winter 1969 0 when a rise occurred A value of 180/120 mmHg was noted in April 1970 before hemodialysis During the hemodialysis period April 1970–January 1973 the blood pressure with few exceptions was within normal limits)

In July 1971 the disc margins were completely blurred with a protrusion of 2–3 diopters No hemorrhages or exudates were present the veins were not distended and the arteries were slightly narrow in appearance Within the papillary tissue some crystalloid formations were seen by the three mirror lens examination indicating that the findings represented a deposit of pathological material within the papillary tissue rather than a true papilledema

Identical changes were found in September 1971 and January 1972 as shown in Fig 1b In September 1971 for the first time a slight reduction in visual acuity was noted as listed in Table I In July 1972 the disc protrusion had almost disappeared as shown in Fig 1c and in December 1972 the left eye suddenly turned amaurotic At this time the disc looked pale and atrophic

3 *Visual fields* The visual fields were normal in 1966 Goldmann s perimetry performed in September 1971 revealed reduced visual field isopters as shown in Fig 2 The visual fields continued to decrease in both eyes (Fig 2) until the left eye turned amaurotic in December 1972 In February 1973 a small central visual field rest was found on the right side

Case 2

The male patient K Aa born in 1918 was diagnosed as having Familial LCAT deficiency in 1973 Since 1944 he has been hospitalized six times because of proteinuria He did fairly well until 19 1 when he developed anemia In 1972 his kidney function started to deteriorate and he probably will soon be a candidate for kidney transplantation The blood pressure was 200/90 mmHg in January and 220/115 mmHg in June 1973

FINDINGS

1 *Corneal opacities* His corneal opacities have been present since early childhood probably since birth The patient has not noted any signs of progression In July 1973 corneal opacities identical to those described in Case 1 were found In addition, some crystals (cholesterol?) were seen peripherally in the corneal stroma near Descemet s membrane at 5–7 o clock The appearance of the right cornea is shown in Fig 3

Papillary manifestations Both discs showed a peripapillary mottled atrophic area



1a



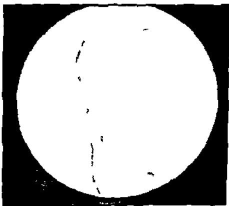
3a



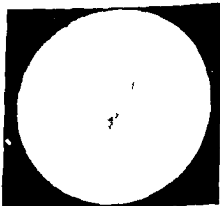
1b



3b



1c



4

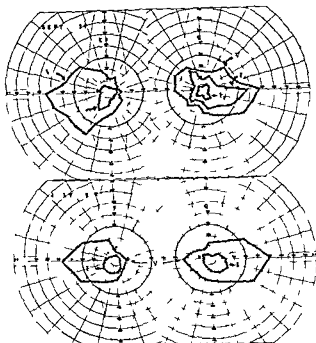


Fig °
Visual fields of Case 1 recorded in September 1971 and July 197 A decrease is noted

Fig 1a
Case 1 May 1971 Retinal hemorrhages at and near the right optic disc.

Fig 1b
Case 1 January 197 Left optic d sc protrusion The crystalloid formations observed within the papillary tissue by the three mirror examination are not visible in this photograph

Fig 1c
Case 1 July 19 Left optic disc atrophy (The corneal opacities may explain the poor quality of these figures)

Fi 3 a and b
R ght m a f Ca e An arcus l poides i p esent (a) and the central part of the cor neal troma how deposit of numerous minute grayish dots (b)

Fig 4
R ght fund s of Case demonstrating ruptures of Bruch s membrane resembling angioid streaks

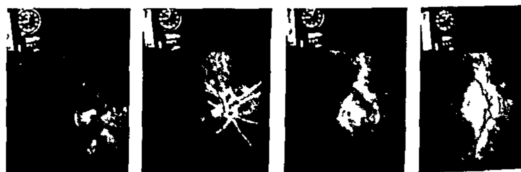


Fig 5
Serial fluorescein fundus photography of Case 2 revealing fluorescence along the streaks suggesting leakage of fluorescein through the ruptured Bruch s membrane (The corneal opacities may explain the poor quality of Fig 4 and Fig 5)

Table I
Deterioration of visual acuity during the follow up period 1971–73 of Case I

Time	Right eye	Left eye
May & July 1971	20/20	20/20
September 1971	20/30	20/60
January 1972	20/30	20/80
June 1972	20/40	20/80
December 1972	20/40	amaurosis
January & February 1973	20/40	amaurosis

with pigmentary accumulations and ruptures of Bruch s membrane (Fig 4) resembling those seen in angioid streaks In accordance with this serial fluorescein fundus photography revealed fluorescence along the streaks and in the mottled peripapillary area (Fig 5) suggesting leakage of fluorescein through the ruptured Bruch s membrane as seen in angioid streaks (Patnaik & Malik 1971)

On the right disc two minor aneurysmatic dilations were seen in a retinal vein On the left side a minute retinal hemorrhage was found close to the disc at 4 o clock No papilledema was found but the retinal surface showed minor areas of grayish appearance close to the optic disc

3 *Ocular function* The visual acuity was normal and the fields were within normal limits in both eyes

4 No lid xanthelasmata were seen

Discussion

The corneal deposits in Familial LCAT deficiency most probably consist of cholesterol or closely related lipid materials From early childhood they give the cornea a characteristic appearance which may be of major diagnostic importance The presence of arcus lipoides in young people is called anterior gerontoxon or arcus juvenilis It may be seen even before puberty in subjects with hypercholesterolemia (Forsius 1954) or in association with other congenital anomalies such as blue sclera megalocornea or aniridia However in these disorders the central part of the cornea is free of deposits The presence of arcus lipoides along with a nebulous appearance of the central part of the cornea should make the clinician think about Familial LCAT deficiency whatever the age of the patient

The granular lattice and macular corneal dystrophies and the crystalline dystrophy of Schnyder may be ruled out as differential diagnostic possibilities because they all look quite different In cystinosis and Hurler s disease the corneal changes may to some extent resemble those seen in Familial LCAT deficiency However the lack of arcus lipoides and the presence of dwarfism make the distinction between these disorders and Familial LCAT deficiency easy The fine dust like opacities in the posterior stroma in cornea farinata and the vaguely defined snowflake opacities in the central cloudy dystrophy of François (François 1956) may resemble the opacities found in the central part of the cornea in Familial LCAT deficiency If cornea farinata or the central cloudy dystrophy of François should occur with arcus senilis the clinical distinction from Familial LCAT deficiency would probably be difficult In Tangier disease (Familial alpha lipoprotein deficiency) the cornea may appear normal (Kocen et al 196) or slightly cloudy (Hoffman & Fredrickson 1965) This slight diffuse opacity can be resolved into fine equidistant dots throughout the corneal stroma presumably due to deposition of cholesterol esters

The fundus changes consisted of aneurysmatic dilations of retinal veins (Case [illegible]) with retinal hemorrhages (both cases) disc protrusion with impaired visual function (Case 1) and ruptures of Bruch s membrane (Case 2) In appearance the disc protrusion of Case 1 to some extent resembled an ordinary papilledema However this was not believed to be due to arterial hypertension, uremia or increased intracranial pressure as no signs of brain tumor were present the blood pressure was fairly normal with only minor and inconsistent elevations and repeated hemodialysis treatments had brought the uremia under control A deposit or leakage of pathological lipid material into the papillary tissue seems a more reasonable explanation This is supported by the observation of crystalloid formations within the papillary tissue and the deterior



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Familial LCAT deficiency – i e increase in membrane cholesterol and lecithin phagocytosis of abnormal lipoproteins by histiocytes formation of foam cells and deposits of pathological membrane surrounded lipid particles in basal membranes and vessel walls

Nervous manifestations have been found in other dyslipoproteinemias such as alpha lipoprotein deficiency (Tangier disease) and beta lipoprotein deficiency (acantocytosis) but have not previously been described in Familial LCAT deficiency

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ation of visual acuity and fields The fields were similar to those found in papillary drusen although no drusen were found

The ruptures in Bruch s membrane of Case 2 may be caused by a malfunction of this basal membrane like structure because of deposits of lipid material An gioid streaks (Doyne 1889) may be found associated with systemic disorders such as osteitis deformans (Batten 1931, Shaffer et al 1957) pseudoxanthoma elasticum (Grönblad 1929) fibrodysplasia hyperelastica of Ehlers Danlos (Cot tini 1949) sickle cell disease (Paton 1959) familial hyperphosphatemia with heterotopic calcification (McPhaul & Engel 1961) idiopathic thrombocytopenic purpura (Yatzkan 1957) and cardio vascular disease with calcification and oc clusion of arteries (Carlborg 1944 Scheie & Hogan 1957) None of these dis orders were found in our cases

The retinal hemorrhages and the aneurysmatic dilations of retinal veins may indicate lesions in the vessel walls as well Deposits of lipid material in the vessel walls may hypothetically lead to disruption of the wall itself with leakage of blood or pathological material into the surrounding nervous tissue causing hemorrhages or disc protrusion as well as obstruction of the artery lumen with ischemic secondary optic atrophy and functional loss This may well be the explanation in our Case 1 Ampulliform dilations and sometimes saccular aneurysms of conjunctival vessels have been found in the here ditary dystopic lipoidosis of Fabry (Rahman 1963) another lipid storage disease Retinal changes which in normotensive patients were limited to the veins included segmental sausage like dilations angulation and tortuosity of the veins In addition to the venous abnormalities arteriolar narrowing A V compression flame shaped hemorrhages and early papilledema presumably as sociated with hypertension may occur in the accelerated phase of the disease (Rahman 1963) Although the retinal changes in Fabry s disease bear some resemblance to those in Familial LCAT deficiency the corneal opacities are different with accumulation of lipid in the epithelial cells leaving the corneal stroma free of deposits

Lipid deposits have been found in many organs in Familial LCAT deficien cy such as cornea plasma membranes (erythrocytes) foam cells in bone mar row and kidneys and in Sea blue histiocytes in bone marrow and spleen All circulating lipoproteins are abnormal in this disease and most of them contain increased amounts of unesterified cholesterol and lecithin Both high and low density lipoproteins are heterogeneous and composed of subfractions in terms of size composition and appearance In both density classes abnormal large molecular weight fractions have been found LCAT is believed to play an im portant part in the homeostasis of membrane cholesterol in general A variety of pathogenetic factors may therefore be at work to explain lipid deposits in

amilial LCAT deficiency – i e increase in membrane cholesterol and lecithin hagocytosis of abnormal lipoproteins by histiocytes formation of foam cells nd deposits of pathological membrane surrounded lipid particles in basal nembranes and vessel walls

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MALIGNANT LYMPHOMA OF THE EYE AND BRAIN

Report of a case

BY

HANS K HARSTAD and KRISTEN ARNESEN

A case of malignant lymphoma (*reticulosarcoma*) of the eye and brain is reported in a 66 year old man presenting clinically as bilateral uveitis In the brain the tumor was confined to the *perivascular spaces* and in the retina tumor tissue was found round the retinal vessels diffusely in the retina in the subretinal space and to a very limited *extent round choroi*dal vessels It is postulated that originally the eye tumor was located in the perivascular spaces of the retina with *secondary breaks* into the other localizations

It is suggested that this is a specific disease *entity different* from the secondary affection of the eye in generalized lymphomas

The nomenclature should be brought into accordance with *the* classification of malignant lymphomas advocated by Rappaport (1966) *and it* is suggested that the condition be designated as *perivascular malignant lymphoma histiocytic type confined to the eye and brain*

Key words tumors – lymphoma perivascular malignant of the eye and the brain

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Primary involvement of the eye with malignant lymphoma or reticulo sarcoma *without evidence of a generalized disease* seems to be rare Only a few cases have been reported in recent years Clinically these patients present signs of uveitis In some cases a similar tumor has been found in the brain

We have observed one case with a malignant lympho reticular tumor of the eye and brain with a clinical history of bilateral uveitis and a rapidly fatal course This may represent a specific disease entity and it is proposed to give it a name which is in accordance with the generally accepted nomenclature for *lympho reticular tumors*

Case History

A 66 year old man applied to the Department of Ophthalmology at the Namdal County Hospital 23 April 1971 complaining of pain and progressive blurring of vision in the left eye for three months and in the right eye for about one month Externally *both eyes were normal except for slight ciliary injection Slit lamp* examination revealed fine keratitic precipitates and flares and cells were observed in the anterior chamber of both eyes The vitreous bodies were cloudy and contained many cells The *optic discs were blurred and edematous more so in the left than in the right eye* No areas of retinal or choroidal involvement were noted at this time Visual acuity 6/5 right eye 6/15 left eye Visual fields were normal Intraocular tension 25 mmHg right eye and *40 mmHg left eye (Schiotz) Clinical* diagnosis *bilateral posterior* uveitis and secondary glaucoma

Corticosteroids were administered topically and systemically and Pilocarpin and Diamox® were given daily In spite of intensive therapy both eyes *continued to fail* The vitreous body of both eyes became progressively cloudy and hazy and a small area of retinal edema with a preretinal exudate was observed temporo superiorly in *the right eye but no further focal chorio retinal lesions or hemorrhages could* be seen on either side

In May 1971 the fundus details were obscured by the vitreous reaction Transillumination revealed no shadows and gonioscopy showed goniosynechiae *In June* 1971 there was no fundus reflex and both eyes were blind with secondary glaucoma Intraocular tension was 40 mm (right) and 50 mm (left) eye

The painful amaurotic left eye was enucleated 15 July 1971 *At that time the* patient s general condition was good General examination and uveitis survey were non contributory and there were no signs of blood disease or lymphadenopathy At the end of July 1971 the condition deteriorated rapidly both *physically and mentally Neurolo*gical examination revealed a neuro myopathy with polyneuritis of the lower extremities and atrophy and weakness of the shoulder muscles on both sides EEG indicated a possible right sided cerebral *lesion Finally the patient became unconscious* and died August 1971 3 1/2 months after he was first seen by the ophthalmologist An autopsy was performed at the county hospital

Pathology

The enucleated left eye was remitted to the Department of Pathology at Ullevål Hospital fixed in 4 % formaldehyde solution The eye measured 25 mm in the anteroposterior and 23 mm in the equatorial diameter The horizontal cups were cut away The retina adhered to the choroid but showed irregular thickenings The vitreous contained gray strands of exudate Horizontal sections through the central part of the eye were stained with haematoxylin + eosin and with Gomori s silver method for reticulin

The anterior chamber was shallow and contained some blood and leukocytes There were peripheral anterior synechiae with complete blocking of the trabecular meshwork In the iris and ciliary body there was a slight inflammatory reaction In the retina there were areas of ischaemic necrosis alternating with edematous areas showing atrophy and hemorrhages (Fig 1) The retinal vessels were congested and in the posterior part including the papilla there were perivascular infiltrates of neoplastic lympho reticular cells (Fig 2) Such cells were also seen more diffusely scattered in the retina A few dilated vessels were completely filled with lipid macrophages

Between the choroid and the atrophic or necrotic retina there was a space of varying width which contained closely packed tumor cells of the same type as those in the retina The cells had scanty cytoplasm and pycnotic or hyperchromatic nuclei of varying shape and size (Fig 3) The bulk of the tumor was situated between the pigment epithelium and Bruch s membrane or rather between layers of the split membrane (Fig 4)

In the choroid proper ... externally to Bruch s membrane there was a more or less heavy infiltrate of inflammatory nature consisting of lymphocytes plasma cells and a few eosinophilic cells (Fig 5) There was no diffuse proliferation of tumor cells in the choroid but in a few places perivascular growth of reticulin positive tumor tissue could be recognized (Fig 4)

A transverse section of the optic nerve showed only atrophy and gliosis

The condition was diagnosed as a malignant lymphoma of the retina and the subretinal space combined with a diffuse uveitis

At the autopsy no abnormal gross changes were noted The remaining right eye was not taken out but tissue specimen from the lungs and the liver as well as the whole brain were fixed in 4 ... formaldehyde solution and sent to the Department of Pathology at Ullevål Hospital Except for thrombo embolic material within the pulmonary arteries and heavy centrilobular congestion of the liver no abnormal change were noted in these organs

In the right parietal lobe of the brain deep in the central white matter there was an area of yellow green discoloration measuring 4 cm ... 8 cm Several pieces from this area were processed for histological examination There was extensive necrosis and a ... of varying size there were cuffs consisting of pleomorphic and atypical histiocytes and ... cells of clearly neoplastic nature (Figs ... & 8) and with a ... network of reticulin fibrils (Fig 9) The neoplastic growth was mainly restricted to the perivascular sheath except for a few minor breaks into the surrounding brain tissue proper The brain tumor consisted of the same type of cells as the eye tumor

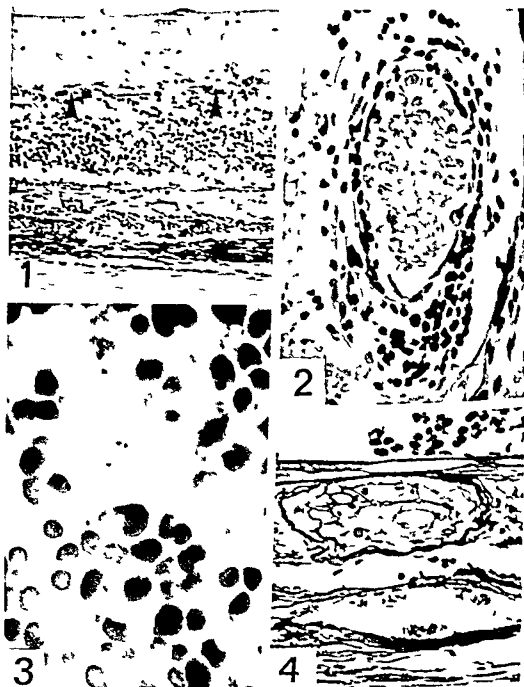
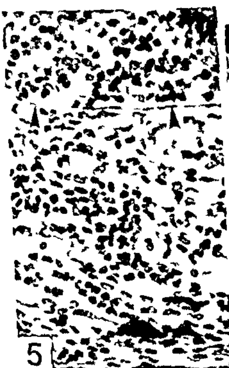


Fig. 1

Section through necrotic retina with scattered tumor cells, subretinal space with closely packed tumor cells, choroid with chronic inflammation and inner part of sclera. Pigment epithelium indicated by black spear heads. Bruch's membrane by white. Haematoxylin and eosin × 180.





Fig

Tumor in subretinal space upper third of picture Choroid with inflammatory reaction lower two thirds Spear heads indicate Bru h s membrane Haematoxylin and eosin × 450

Fig 6

Under degenerated pigment ep thelium (arrows) a broad band of tumor cells in sub ret nal space Bruch s membrane appears as a pale indistinct zone (spear head) Haematoxylin and eosin 1100

Fig

Perivascular tumor infiltrat n in poster or part of retina Haematoxylin and eosin 450

Fig 3

Tum r cells in subret nal space Haematoxylin and eosin 1100

Fig 4

Reti ul n positive perivascular tumor infiltration in choroid Comori s silver impregna tion 450

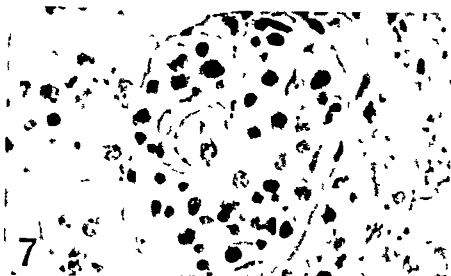


Fig 7

Perivascular extension of tumor tissue in the brain Haematoxylin and eosin × 450

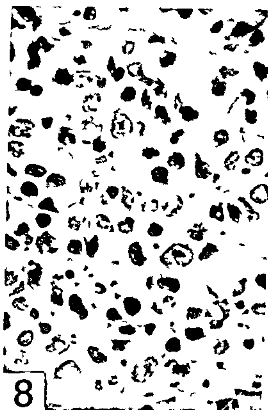




Fig 8

Perivascular space of larger vessel than in Fig 7 Infiltration of atypical pleomorphic histiocytes and reticulum cells Haematoxylin and eosin × 450

Fig 9

Vessel of same size as in Fig 8 Reticulin positive tumor tissue in perivascular space Gomori's silver impregnation × 450

Discussion

A malignant neoplasm of lympho reticular type was found in one eye and in the brain of a 60 year old man without evidence of other organs being affected except probably the second eye

A more generalized type of lymphoma can not be excluded in a case like this without detailed histological studies of the lymph nodes spleen and bone marrow The clinical examination and the gross findings at autopsy did not give any evidence of a wide spread disease of the lympho reticular system and therefore we have postulated that the neoplastic process did not extend beyond the eyes and the brain

The tumor in the brain was located in the perivascular spaces In the eye the main bulk of the neoplasm was found in a wide gap between the pigment epithelium and Bruch s membrane or between two layers of the membrane There was in addition perivascular extension of tumor tissue in the retina (Fig 7) and we think that this represents the primary location of the tumor within the eye and that the subretinal tumor mass as well as the perivascular cuffs of tumor in the choroid (Fig 4) represent a secondary spread Thus the tumor in the eye and the tumor in the brain can both be regarded as a malignant neoplasm of the perivascular mesenchyme in the central nervous system

The central nervous system including the retina does not contain mesenchymal tissue except for the blood vessels with their perivascular sheaths and the microglia which is supposed to enter the central nervous system along the blood vessels (Bloom & Fawcett 1962) In the perivascular space there are *mesenchymal* cells including the pericytes (adventitial cells Rouget cells) which are considered to belong to the reticulo endothelial system and thus to be closely related to histiocytes and reticulum cells with corresponding functional potentialities (Stöhr v Möllendorff Goertler 1959) *Pericytes* are also found in the walls of the retinal capillaries (Hogan Alvarado & Weddell 1971)

The most comprehensive discussion of sarcomas of the brain was given by Kernohan & Uihlein (1962) In their book a whole chapter is devoted to sarcomas of the reticuloendothelial system Whereas British authors have preferred the designation microglioma the Americans favor the diagnosis of reticulo endothelial sarcoma Many other names have been suggested by various authors There is general agreement that these tumors are rare in the brain Kernohan & Uihlein presented 40 *cases* 12 of which were autopsied In two of these extracerebral tumors of the same type were found but systematic studies of the eyes do not seem to have been carried out In 28 surgical cases the tumors were restricted to the brain except for one case with lung metastasis

The frontal lobe was most frequently affected and multiple or diffuse localizations were frequent. The majority of cases occurred in the first, fifth and sixth decades. Reticulin production was evident in all tumors and the relation of the neoplasm to the walls of blood vessels was strongly emphasized, but breakthrough of the tumor through the pia glia barrier was evident, especially in the central parts of the tumors.

Under the designations "primary reticuloendothelioses" or "neuroreticuloses" respectively, Wilke (1950) and Draganescu & Vuia (1965) discussed cerebral lesions corresponding closely to the brain tumor of our case and pointed out that these may be either of inflammatory or neoplastic nature. The criteria for this distinction were not too clearly defined, however, and it is often very difficult to draw a sharp line of demarcation between these two categories.

The combination of a malignant mesenchymal tumor of the eye under the clinical picture of uveitis with a similar tumor of the brain was described by Vogel, Font, Zimmerman & Levine (1968). They studied seven eyes from six patients, five of whom presented with clinical uveitis of unknown cause. Histopathologically, a reticulo sarcoma of the uvea or retina or both was demonstrated in all eyes, together with a moderately severe chronic uveitis of non-granulomatous type. The tumor tissue was distributed perivascularly. In two of their patients a similar neoplasm was found in the brain, just as in our case. It was concluded that reticulum cell sarcoma may originate in the ocular tissues without being part of a generalized disease.

One of the cases in this series had been provided by Dr. C. Naumann in Hamburg, who presented another case of the same type at the 1973 meeting of the European Ophthalmic Pathology Society in Dublin, where our case was also demonstrated.

Neault, Van Scoy, Okazaki & MacCarty (1972) presented 17 patients from the Mayo Clinic between 1956 and 1968 with reticulo sarcoma of the brain. Seven of these patients had clinical uveitis. Histological examination of the eyes could not be carried out, but the authors concluded that the underlying condition in the eyes with uveitis might be reticulo sarcoma, just as in the cases reported by Vogel, Front, Zimmerman & Levine (1968).

Rappaport (1966) proposed a simplified classification of malignant lymphoreticular neoplasms which we have adopted in a sligthly modified form:

Malignant lymphoma, lymphocytic type, nodular or diffuse
Malignant lymphoma, histiocytic type, nodular or diffuse
Malignant lymphoma, mixed type, nodular or diffuse
Burkitt's tumor
Hodgkin's disease
Others

According to this classification it is proposed to designate the neoplastic condition described in this paper as *perivascular malignant lymphoma histiocytic type confined to the eye and brain*

We believe that this represents a disease entity which may not necessarily be as rare as appears from the literature The condition may be overlooked as most routine autopsies do not include the eyes even if there is information of eye disease in the clinical record

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ELECTRO-OCULOGRAPHY IN ALBINOS

BY

D K GAHLOT and E HANSEN

EOG recordings in groups of 30 normal Caucasian subjects six normal Negroes and six albinos have been evaluated Compared with the normal Negro and Caucasian subjects the albinos showed a generally low base value for the EOG amplitudes a delayed rise in the light phase and an earlier occurrence of the dark trough whilst the EOG ratio itself was unaffected

Key words albinos – EOG ratio – light peak – dark trough – time relations

Since the demonstration of an electrical potential in vertebrate eyes in 1849 by Du Bois Reymond several authors have studied the variations of the standing potential in normal and pathological cases (Marg 1951 François Verriest & de Rouck 1955 Kris 1958 Arden Barrada & Kelsey 1962) It is uncertain as to what degree the natural abundance of pigments in the patient s eyes influences the recording of electro oculography (EOG) It is the purpose of this paper to examine the differences in the EOG recordings of groups of normal Caucasian subjects normal Negroes and albinos

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Material and Methods

The normal material comprizes 30 Caucasian subjects of Scandinavian origin and six African Negroes Their mean age was 28 years with a range from 16 to 45 years They all had normal vision in each eye with or without correction The eye examination revealed no pathological changes The albinic group comprizes six patients with complete albinism These patients had poor visual acuity and nystagmus An additional albinic patient had to be excluded because of pronounced photophobia and nystagmus The method of registration was essentially the same as used by Arden Barrada & Kelsey (1962) The patient was seated comfortably in a chair with the head rest positioned 1 m from an opalescent screen illuminated from the rear by fluorescent tubes The illuminance measured at the patient s head was approximately 500 lux The patient was instructed to alternate fixation between two red lights attached to the screen with an angular distance of 35 The regularity of the eye movement was controlled by the clicks of a metronome kept at a constant speed (about 70 per min) The patient was told to move his eyes only when the metronome clicked In this way short periods of regular oscillations were registered with intervals of 0 5 to 1 min This procedure allowed the patient sufficient rest in between However the patient was specially instructed to keep his eyes open and fixed towards the screen during the entire period of examination in spite of discomfort The patient was prepared in an ordinarily illuminated room followed by a pre adaptation period of 15 min in darkness The registration started during the last [illegible] 3 min of this period Then the screen was lighted and the recording continued for 15 min after which the light was switched off again and the recording was continued in total darkness for another period of 15 min before the examination was finished The examination was performed with normal pupils without the use of mydriatics Both eyes were used for recording Five mm silver electrodes were attached on each side of the eyes near the canthus internus and externus The two eyes were connected to separate channels of the Mingograph (Elema Schonander) Indifferent electrodes were attached to the cheek

Results

The variation in amplitude throughout the registration were measured and the ratio LP DT 100 as given by Arden Barrada & Kelsey (1962) was calculated LP indicates the light peak and DT the dark trough Values of 185 or above were regarded as normal Typical recordings obtained by the three groups of

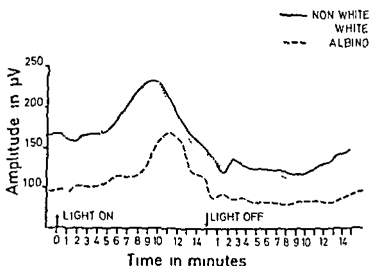


Fig 1

Variations in amplitude during adaptation to light and darkness in three typical representatives of non white white and albinic patients

patients are shown in Fig 1 Even though a single base value does not give *much information* about pathological significance as it is influenced by several *accidental* circumstances such as the placing of the electrodes etc it is noticed *that a low* base value is a general trend among the albinic patients The mean

Table I

The mean values of data registered by EOG for the three groups of subjects

	Normal Caucasians	Normal Negroes	Albinos
No of subjects	30	6	6
Base value	147 17	146 67	84 58
s d	56 48	22 19	32 55
Ratio LP DT × 100	225 10	218 00	229 00
s d	34 02	22 62	35 59
Time/light peak (T_1)	8 53	9 03	10 13
s d	0 93	0 82	0 70
Time/dark trough (T)	9 19	9 75	8 04
s d	0 99	0 76	0 51
T_1 + T	15 02	15 77	18 17
s d	1 41	0 82	0 86

base value in the albinic group is significantly lower than in the two normal groups ($P < 0.01$) Table 1 shows the results of the EOG recordings expressed by mean values in the three groups The average ratio LP/DT x 100 is on a fairly constant level for the three groups although there are wide individual variations as expressed by the standard deviations The differences are not significant ($P > 0.10$) The time for reaching the light peak does not differ significantly between the two normal groups but is significantly delayed for the albinic group in relation to both normal groups ($P < 0.01$) Likewise the two normal groups do not differ significantly in their time for reaching the dark trough whilst the albinic group on an average reaches the dark trough significantly earlier than the other two groups ($P < 0.01$) When the time for reaching the light peak and the time for reaching the dark trough are added there is however a marked tendency for an adjustment to a constant value The mean values do not differ significantly ($P > 0.10$)

Comments

There was a slight degree of irregularity in the curves showing the variations of the amplitudes during the light and dark phase (Fig 1) We noticed that each time the panel light was put on there was a short lasting drop in amplitudes within the first few minutes of the light phase Likewise there was a short rise in amplitudes at the beginning of the dark phase These slight adverse effects just after the changing of the adaptational state may indicate the presence of several components in the standing potential Kris (1958) described similar short lasting initial transient effects of the eye potential level

Why the albinic patients in general have a lower base value and show other time relations to the light peak and the dark trough in their EOG than the normal subjects is difficult to explain It might possibly be due to a more direct action of the light on the pigment epithelium in the eyes of albinos because of the lack of shielding pigmentation in the anterior parts of their eyes It has also been shown that the lack of pigmentation in albinic animals influences several parameters of the ERG Reuter (1973) found shorter latency of the *a* and *b* waves but longer latency of the *c* wave of the ERG in albinic rabbits than in pigmented animals

As no significant difference was found between our three groups of patients concerning the ratio LP DT x 100 it has been proved true that it is the functional competence of the pigment epithelium and not the amount of melanotic

pigments which is of importance in the EOG recording A supernormal response to the EOG in albinos was stated by Reeser et al (1970) However Gouras & Gunkel (1963) found a normal EOG ratio which is consistent with the finding in our series

Acknowledgement

We wish to thank Professor Peter Berdal Head of the E N T Department Rikshospitalet Oslo for his kindness in placing room and equipment at our disposal in the E N T Department

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URINARY METABOLIC STUDIES IN HEREDITARY MACULAR DEGENERATION

BY

G HOLMGREN S NORDSTRÖM and W THORBURN

Urinary metabolic studies by means of high voltage paper electrophoresis (HVPE) and some qualitative chemical tests were performed in 40 patients in a family with hereditary macular degeneration and in 40 *nonaffected members of the same family* No biochemical abnormalities were observed in the examined urine samples The results are discussed with regard to earlier published reports of aminoaciduria in hereditary macular degeneration

Key words hereditary macular degeneration – urinary metabolic studies high voltage paper electrophoresis

Introduction

In 1905 Best in Germany presented a pedigree with 8 cases of *congenital hereditary macular degeneration* among 59 persons examined Since then several other pedigrees have been presented in both European and American literature and have been reviewed in comprehensive surveys (Braley and Spivey 1964 Waardenburg 1963) In Sweden surveys have been made by Barkman (1961) and by Nordström et al (1972)

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There have been many problems concerning the classification of hereditary degenerations or dystrophies of the macula Cogan (1965) proposed that a meaningful system of classification should be based upon the metabolic or biochemical abnormality involved as in Tay Sachs disease Basen Kornzweigs disease and Refsum s disease Bessman & Baldwin (1962) reported a family with cerebromacular degeneration associated with dipeptiduria and imidazole aminoaciduria but according to Lefler et al (1971) the ocular disorder in this report was actually a retinitis pigmentosa like retinal degeneration and not a true macular degeneration

Only a few studies concerning the association between hereditary macular degeneration and aminoaciduria or other metabolic abnormalities have been presented Lefler et al (1971) studied urinary amino acid excretion in a large family by means of thin layer electrophoresis According to these authors there was a prominent association between the disease and the presence of varying degrees of generalized aminoaciduria as well as glycine and histidinuria The present investigation was an attempt to confirm their results A large family with hereditary macular degeneration was studied from the genetic point of view Further studies are planned to analyze possible disorders connected with the macular lesion

Material and Methods

The family has previously been presented in part by Nordstrom et al 1972) Several other affected members have since been identified and we have found a connection between pedigrees one and two (Full details of this family will be presented elsewhere Nordstrom (1974)) From pedigree 1 80 members were available for urinary metabolic examination Of these 40 had macular degeneration all of them had been examined at different departments of ophthalmology At the University of Umeå 36 members were examined and the diagnosis was used on characteristic changes of the macular region which were similar to those described by Lefler The remaining 40 subjects studied had unimpaired vision 28 of these underwent the same type of ophthalmological examination as the affected members above and showed no macular lesions No alteration or control of the subjects diet was performed

Urine samples were collected as overnight urine and were kept at –25° C in the laboratory until analysis

The metabolic studies included the following tests to detect abnormal amounts of certain amino acids and carbohydrates

1 High voltage paper electrophoresis (HVPE) for the separation of amino acids performed at pH 1 9 according to Holmgren et al (1970) 2 *Albustix*™ (AMES) for the detection of albumin 3 Phenistix® (AMES) for the detection of phenylketones 4 The cyanide nitroprusside reaction (Brand s test) for the detection of cystine and homocystine Brand *et al* (1930) 5 Gerbers test for the detection of increased amounts of histidine Gerber & Gerber (1969) 6 Clinistix® (AMES) for the detection of glucose 7 Clinistix tablets® (AMES) for the detection of reducing substances 8 The nitrosonaphthol reaction Perry et al (1968) for the detection of tyrosine metabolites 9 The dinitrophenyl hydrazine reaction Perry et al (1968) for the detection of ketoacids

If there were positive reactions in the tests 2 9 an internal comparison of all test results were performed and compared to the HVPE pattern Transient positive tests or abnormal patterns at HVPE were checked by analyzing fresh urinary samples Pathological patterns at HVPE was analyzed by ion exchange chromatography The evaluation of these screening tests is described by Hambraeus & Holmgren (1974) and Holmgren (1973) A high degree of reliability can be attained with the tests used in this study The evaluation regarding a possible hyperaminoaciduria is thereby based on an internal comparison of the different test results By means of these chemical procedures several metabolic disorders resulting in an overflow or renal hyperaminoaciduria can be detected

Results

A normal pattern on HVPE and negative reactions in all qualitative chemical tests were found in all urine samples

Discussion

The prominent association between aminoaciduria and macular degeneration observed by Lefler et al (1971) has not been confirmed in this study as no pathological excretion of amino acids could be demonstrated The description of the clinical signs the age of onset and the development and appearance of the fundus alterations in the material of Lefler et al indicates that they were dealing with an eye disease similar to that in the family we have studied

Lefler et al also report another family in which there was no evidence of aminoaciduria under conditions of normal food intake however when fasted the affected individuals showed a distinct hyperaminoaciduria of prolin and hydroxyproline In the present study no attempt was made to alter or control the subjects diet According to Holmgren (1974) however dietary protein intake has little effect on the urinary excretion of amino acids In spite of a sometimes very long time interval between the preceding meal and the collecting of the urinary samples no evidence was ever found of excretion of hydroxyproline or proline

The different results obtained in the above study as compared to the present result might be due to different phenotypic variants of hereditary macular degeneration

The hypothesis proposed by Lefler et al (1971) concerning a connection between macular degeneration and aminoaciduria is attractive If such a connection exists patients with aminoaciduria but without fundus alterations might be identifiable as future victims of macula degeneration This would make it possible to trace subclinical expression of the gene causing macular degeneration by HVPE examination of urine However there are objections to this hypothesis It is rather difficult to imagine the mechanism behind a normoaminoacidemic hyperaminoaciduria which could expl in connection with an ophthalmological condition It is also difficult to evaluate the significance of a slight generalized or even a pecific hyperaminoaciduria e g histidinuria or glycinuria especially when analyses by ion exchange chromatography have not been performed Furthermore hyperaminoaciduria may be intermitt nt due to such factors as hormonal changes (Soupart 1962) dietary intake (Hubbard & Block 196) or medication (Mozziconacci et al 1964) In addition inconsistent test results may be due to technical difficulties (Holmgren 19 and Holmgren 1974) However increased excretion of histidine or glycine due to defective renal reabsorption of these amino acids seems to occur according to Holmgren et al (1974) and Schreier (196)

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EARLY EFFECTS OF EPINEPHRINE AND PILOCARPINE ON THE INTRAOCULAR PRESSURE AND THE EPISCLERAL VENOUS PRESSURE IN THE NORMAL HUMAN EYE

BY

KENNERTH WILKE

Topical instillation of one drop of epinephrine 1 % and pilocarpine 2 % in normal human eyes were found to give a *transient* increase of the intraocular pressure (IOP) and the episcleral venous pressure (P_{ev}) of about ? to 5 5 mmHg The maxima were reached *in 5 to 10* minutes after instillation During the rising phase the change of the P_{ev} preceded that of the IOP

Key words intraocular pressure – episcleral venous pressure epinephrine – pilocarpine

Introduction

In ophthalmology epinephrine and pilocarpine topically applied are widely used because of their effects on the intraocular pressure (IOP)

The decreasing effect of *epinephrine* on IOP depends on a reduction of the rate of aqueous humor inflow (Weekers et al 19?4) but there is also an increase in the outflow facility both *in* man (Langham et al 19 1) and in the vervet monkey (Bill 1969) However some investigators have found a transient rise in the IOP soon after application of epinephrine in man (Poos 1931 Lee 1958)

Rece ved December 7 19 3

This effect which is also seen in the rabbit (Norton et al 1972) is attributed to a decrease in the outflow facility in this animal The first effect of epinephrine to appear when topically applied is a vasoconstriction which affects primarily the superficial conjunctival vessels but also the deep episcleral vessels There does not seem to be a *significant effect of epinephrine on the* aqueous flow in man which may be explained by the fact that no capillaries are found between Schlemm s canal and the aqueous veins (Gazala et al 1968) In normal human eyes a decrease in the intravascular pressure of recipient veins (P_{rv}) after epinephrine instillation has been reported (Löhlein 1950) but in the vervet monkey the epinephrine tends to raise the P_{rv} (Bill 1969)

The IOP decreasing effect of *pilocarpine* seems to depend on an increase in outflow facility which is found in monkey (Ceropithecus ethiops) (Barany 1962) as well as in man (Krill & Newell 1964) but a decrease in aqueous formation is also observed in monkeys (Macaca irus) (Bill & Wålinder 1966) The pressure decreasing effect in normal human eyes is significant 60 min after instillation but is preceded by a transient pressure increase (Lohlein 1950 Lenton & Schwartz 1963) Immediately after instillation of pilocarpine there is a vasodilation of the conjunctival vessels and an increase of blood in the aqueous veins (Ascher 1961) A transient increase of the P_{rv} is observed in the vasodilation period (Löhlein 1950)

Both epinephrine and pilocarpine affect the conjunctival vessels within one or two minutes after local administration Part of the drugs will penetrate into the eye and in a few minutes will influence the iris the ciliary body and the aqueous production Thus in the rhesus monkey pilocarpine is found in the aqueous within five minutes after topical administration (Asseff et al 1973)

In a previous paper (Krakau & Wilke 1973) the possible interaction between the venous and intraocular pressure was discussed The aim of the present investigation is to further elucidate these relations by recording the IOP and the P_{rv} after pharmocological disturbances of the superficial conjunctival circulation The main interest lies in the time relations between IOP and P_r during the first few minutes after instillation of epinephrine and pilocarpine

Material and Methods

Material

The measurements were made on healthy young women aged 20 35 All of them were familiar with the test situation The IOP series were made on four subjects three of whom had a distinct recipient vein and could also be used for the venous pressure series

Measurements of the IOP

The measurements of the IOP were made by the vibration tonometer (Krakau 1970) on seated subjects After local anaesthesia with Novesin 0 4% the plunger of the vibrometer was left on the cornea for 5 10 secs in every measurement The load of the tonometer was 0 3 g

Measurement of the episcleral venous pressure (P_v)

The episcleral venous pressure was measured by the instrument described by Krakau Widakowich & Wilke (1973) An air jet was used to compress the vessel and the pressure of the air stream was raised until total obstruction of the vessel was seen (= +++ effect) This +++ value was higher than the true venous pressure The measurements were made on so called recipient veins i e an episcleral vein which has just received a large aqueous vein and in a few cases also on conjunctival veins As a flagrant sign of total obstruction of the recipient vein was taken a reversed flux of aqueous into an afferent branch of the episcleral vein or an influx of blood into the aqueous vein All measurements were made on seated subjects and local anaesthesia was used as in the IOP measurements

Experiments and Results

Measurements of the IOP

In this series of four subjects every experimentee was subjected to three sub series

A Control series with measurements at one to one and a half minute intervals for twenty minutes

B After two or three measurements showing no trend one drop of epinephrine 1% (Eppy®) was instilled in the eye topically and measurements were then taken at one to one and a half minute intervals for twenty minutes Another two or three measurements were taken forty and sixty minutes after the instillation of the epinephrine

C This series was performed like series B but pilocarpine 2% was instilled Series A B and C were repeated two or three times (Table I) in random order but only once a day in every subject and the mean curves from each subject are given in Fig 1

This effect which is also seen in the rabbit (Norton et al 1972) is attributed to a decrease in the outflow facility in this animal The first effect of epinephrine to appear when topically applied is a vasoconstriction which affects primarily the superficial conjunctival vessels but also the deep episcleral vessels There does not seem to be a significant effect of epinephrine on the aqueous flow in man which may be explained by the fact that no capillaries are found between Schlemm s canal and the aqueous veins (Gazala et al 1958) In normal human eyes a decrease in the intravascular pressure of recipient veins (P_{rv}) after epinephrine instillation has been reported (Löhlein 1950) but in the vervet monkey the epinephrine tends to raise the P_{rv} (Bill 1969)

The IOP decreasing effect of *pilocarpine* seems to depend on an increase in outflow facility which is found in monkey (Ceropithecus ethiops) (Barany 1962) as well as in man (Krill & Newell 1964) but a decrease in aqueous formation is also observed in monkeys (Macaca irus) (Bill & Wålinder 1966) The pressure decreasing effect in normal human eyes is significant 60 min after instillation but is preceded by a transient pressure increase (Lohlein 1950 Lenton & Schwartz 1963) Immediately after instillation of pilocarpine there is a vasodilation of the conjunctival vessels and an increase of blood in the aqueous veins (Ascher 1961) A transient increase of the P_{rv} is observed in the vasodilation period (Lohlein 1950)

Both epinephrine and pilocarpine affect the conjunctival vessels within one or two minutes after local administration Part of the drugs will penetrate into the eye and in a few minutes will influence the iris the ciliary body and the aqueous production Thus in the rhesus monkey pilocarpine is found in the aqueous within five minutes after topical administration (Asseff et al 1973)

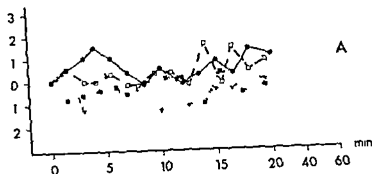
In a previous paper (Krakau & Wilke 1973) the possible interaction between the venous and intraocular pressure was discussed The aim of the present investigation is to further elucidate these relations by recording the IOP and the P_r after pharmocological disturbances of the superficial conjunctival circulation The main interest lies in the time relations between IOP and P_r *during* the first few minutes after instillation of epinephrine and pilocarpine

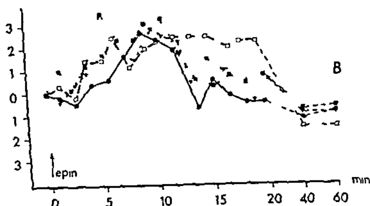
Material and Methods

Material

The measurements were made on healthy young women aged 20 35 All of them were familiar with the test situation The IOP series were made on four subjects three of whom had a distinct recipient vein and could also be used for the venous pressure series

IOP





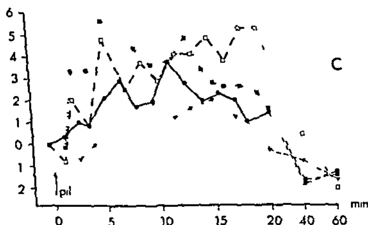


Fig 1

IOP (v brometer) from four individuals A control *series* B topical application of one drop of epinephr ne 1 % C topical applicat on of one drop of pilocarpine ? / Each curve s the mean of two or three experiments The initial values are made equal to zero for all *experimentees* The absolute initial pressure values are given in Table I The arrows ind cate the time *for drug* application

Table I
Mean initial pressure values (mmHg)

	IOP			+++		
	A	B	C	A	B	C
G W	19.1	18.5	18.8	16.5	15.6	15.9
G P	11.9	12.7	13.1	16.7	15.3	15.7
C N J	16.8	15.2	16.5	17.0	17.0	15.5
B R	11.9	12.1	11.5			

The IOP in the control series showed more or less pronounced fluctuations but no significant trend. In series B there was a transient increase in the IOP of 2.5 to 3.5 mmHg in all cases following instillation of epinephrine (Fig. 1 B). Forty and sixty minutes after the instillation of epinephrine the IOP was lower than before the instillation in all cases. In each series there was an impressive vasoconstriction of the conjunctival vessels within the first minute after the instillation of the epinephrine, which lasted more than sixty minutes. Occasionally there was a very slight mydriasis forty and sixty minutes after the instillation of epinephrine.

In all experiments in series C there was a marked vasodilation soon after instillation of pilocarpine and a miosis after ten to fifteen minutes. There was a transient increase in IOP of 2 to 5.5 mmHg. The IOP was reduced as a rule forty and sixty minutes after instillation in all cases (Fig. 1 C).

In both series B and C there was a pressure increase in all cases 10 minutes after drug instillation. For a significant test the difference between the value at 10 minutes and the initial value P_{10} P_0 was calculated for series A, B and C. The range and mean of these differences are given in Table II. The significance of the increase has been tested by considering the mean drug effect of an individual and the mean in the corresponding control series as paired observations. By means of the t test we find the pressure increase in series B and C significant on the 99.8 % level.

Measurements of the episcleral recipient vein pressure P_r

In this series of three subjects from the previous series the P_r was measured at two minute intervals. A two minute interval was chosen because a one minute interval was found to give a conjunctival irritation at the end of the series

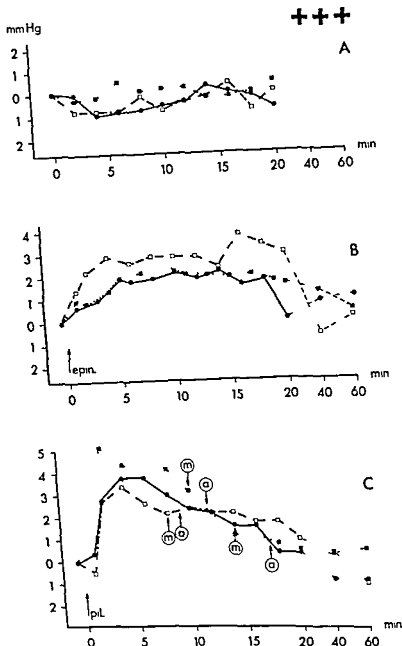


Fig 9

Measurements of the I_{ry} and topical application of one drop of epinephrine 1% • and p locarp ne % • in three subjects In C, *m* = means the time for appearance of miosis and *a* = for reappearance of aqueous

Table II
P_{10} P_0

	IOP									+++								
	A Control			B Epinephrine			C Pilocarpine			A Control			B Epinephrine			C Pilocarpine		
	R	m	n	R	m	n	R	m	n	R	m	n	R	m	n	R	m	n
●—— G W	1 6	0 5	3	0 4	2 2	3	0 8	2 7	3	0 6	-0 [illegible]	3	0 8	2 2	3	0	2 4	2
□- - - G P	1 6	0 3	3	0 8	2 2	3	2 4	2 8	2	1 6	-0 8	2	0 8	2 8	2	0 4	2 6	2
■ - - - C N J	2 2	0 1	3	1 5	3 0	3	1 5	3 7	2	1 2	0 1	3	1 2	2 5	3	0 4	3 0	2
▲- - - - B R	2 1	-1 5	3	1 6	2 4	3	0 8	2 0	2									

R range m = mean n = number of experiments
I_0 = first value in the series I_{10} = value after 10 min

Following the instillation of epinephrine there is a vasoconstriction of the epibulbar vessels a reduction in the proportion of blood and a lower flow rate in the recipient vessel The aqueous veins do not seem to be involved in the vasoconstriction and the aqueous flow seems to continue Due to a general constriction of the episcleral veins there is an increased resistance to the flow from the recipient veins The continuous flow of aqueous might then contribute to the fairly slow increase of P_{rv}

On the other hand pilocarpine causes a dilatation of the conjunctival and episcleral vessels and an increased proportion of blood in the recipient veins Arterio venous shunts have been described in the episcleral vessels of the Rhesus monkey (Gaasterland et al 1970) and in the human conjunctiva (Grafflin & Corddry 1953) The fast pressure reaction after pilocarpine might well be explained by an opening of such shunts

Could then the rise in IOP be secondary to the rise in P_{rv}? If we presume that the aqueous production and the outflow resistance remain unchanged an increase of P_{rv} gives an augmentation in IOP but due to the windkesseleffect (the bulb volume increasing with increased pressure) a time lag between P_{rv} and IOP changes has to be expected

If we plot the differences between the IOP and the P_{rv} curves in series B and C and make the initial values equal we should expect a negative phase during the rising period of the P_{rv} corresponding to the blowing up of the bulb Later when P_{rv} is decreasing after passing a zero point where the P_{rv} and the IOP are equal a positive phase should follow when the bulb is deflated As mentioned both pilocarpine and epinephrine penetrate the bulb rapidly and only during

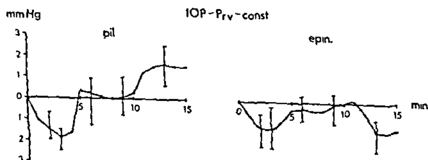


Fig 3
The difference between the IOP and the P_{rv} curves (mean of three subjects Figs 1 & 2) after application of pilocarpine and epinephrine The initial values are made equal to zero The range is marked by vertical lines

Three subseries corresponding to series A, B and C in the IOP measurement series were made and repeated in random order two or three times (Table II) but only *one series was made every day in each subject*. The mean curves are given in Fig. 2.

In the control series the fluctuations of the episcleral venous pressure were slight and no significant trend was found (Fig. 2). At the obstruction point in the recipient vein (+++) the blood in the episcleral vein passed into the aqueous vein.

In series B there was an obvious vasoconstriction of the conjunctival vessels as in the IOP measurement series. Aqueous filled most of the recipient vein which seemed to be of normal width, whereas the blood stream in these veins diminished and the flow rate of blood strikingly slowed down for more than twenty minutes. A transient increase in the P_{rv} of 2.5 to 3.5 mmHg was noted in all subjects (Fig. 2). At the obstruction of the recipient vein (+++) there was an influx of aqueous humor from the aqueous vein into the episcleral vein. During some parts of this series it was difficult to get reliable values because of *the lack of blood in the recipient veins*.

In series C there was a marked vasodilation in the conjunctival and episcleral vessels within one or two minutes of the instillation of pilocarpine and this effect lasted about twenty minutes. Even the aqueous vein was *completely* filled with blood and there was an increase in the P_{rv} of 3.5 to 5 mmHg. When testing the P_{rv} increase (Table II) in analogy with the IOP series we found the pressure increase in series B and C at 10 minutes to be significant on the 99.95% level. The conjunctival venous pressure was measured in a few cases and showed a similar increase. After nine to seventeen minutes a visible flow of aqueous reappeared in the recipient veins and the P_{rv} was normalized (Fig. 2).

It would have been an advantage to measure both the IOP and the P_{rv} alternately in the same experiment, especially for the study of the difference between the IOP and the P_{rv}. At present this cannot be done with the subject seated at the same instrument during the whole series, which is why we had to refrain from this experimental design.

Discussion

The effects of epinephrine and pilocarpine locally administered on the conjunctival and episcleral vessels are very striking but completely different. Nevertheless, in both cases an increase in P_{rv} is provoked. We can hardly attribute this to the IOP increase since the latter starts somewhat later than the *increase in* P_r.

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the first 5–10 minutes can we hope that the presumed constancy of outflow facility and aqueous production holds In Fig 3 the IOP – P_{rv} differences have been plotted For the pilocarpine experiments the negative and positive phases are clearly discernible Also for the epinephrine experiments the negative phase is obvious but the pressure effects in this case are more protracted and a positive period is not observed which might be attributed to altered flow or resistance Nevertheless the findings made do not militate against the view that the initial IOP changes are secondary to the venous pressure changes in these experiments

This study which has shown that changes in the conjunctival vessels could influence the P_{rv} and the IOP will also support our previous assumption (Krakau & Wilke 1973) that the conjunctival vessels may play a role in regulation of the IOP With pilocarpine we obtained a rise of the P_{rv} and the IOP by about 4 mmHg and by merely loading the eye with 2 5 g there was a pressure decrease of the same order That means that by simple actions we are able to influence the IOP via the P_{rv} by no less a range than 8 mmHg It does not seem improbable to us that some of the changes reported by tonographers in the outflow facility might be attributed to venous pressure changes

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The muscles of the first class are termed multiple unit muscles the second syncytial muscles

The dilator muscle of the pupil belongs to the multiple unit class This can be demonstrated very easily If a small quantity of epinephrine is injected subconjunctivally or applied by means of a cotton wool pledget to the anesthetized conjunctiva the pupil is deformed by a localized contraction of the dilator muscle

There is little doubt about the place of the ciliary muscle in this classification it is a multiple unit muscle There is morphologic basis for believing that the human ciliary muscle is composed of physiologic units i e bundles of several muscle cells These aggregates separated from each other by fibroblasts contain nerves and nerve endings which may be related to more than one cell but only to those of one bundle (Ishikawa 1962)

Experimental evidence has been presented by Barany and Rohan (1965) concerning the ciliary muscle of the vervet monkey Ceropithecus ethiops If it is possible to achieve a localized concentration of the ciliary muscle in man by local application of pilocarpine this might verify the observations of Barany and Rohan and at the same time give some information about the mechanism of accommodation

Pilocarpine drops applied in the usual way in the conjunctival sac give miosis and in a young individual myopia The pilocarpine will be diffused all over the conjunctiva by blinking The concentration of pilocarpine and the resulting contraction of the ciliary muscle will be about the same all around the circumference of the muscle This might conceivably result in astigmatism if there is an asymmetry of the ciliary muscle e g if a sector of the muscle is atrophic and weaker than the rest Also the miosis might cause astigmatism if the most axial part of the surface of the lens differs in curvature from that in the more peripheral parts of the normal pupil

If the pilocarpine is applied locally with a cotton wool pledget to the anesthetized conjunctiva a certain spread is unavoidable on the surface of the conjunctiva during the passage through the conjunctiva and sclera and also in the ciliary body It is not possible to achieve a localized contraction of the ciliary muscle but only a certain asymmetry of contraction An asymmetric contraction will give lens astigmatism but it is not possible to predict the position of the axis of astigmatism If for example the contraction is limited to a quadrant of the circumference of the muscle the axis of positive astigmatism will be at right angles to the meridian of application If more than half of the circumference of the ciliary muscle is contracted this axis will be parallel to the meridian where the pledget of pilocarpine is applied

After some preliminary trials to find an adequate method of application and

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ASTIGMATISM OF THE LENS BY ASYMMETRIC CONTRACTION OF THE CILIARY MUSCLE

BY

ULF HALLDÉN and MARIANNE HENRICSSON

A solution of pilocarpine was applied with a cotton wool pledget to the surface of the bulbar conjunctiva This resulted in astigmatism by asymmetric contraction of the ciliary muscle This seems to confirm that the ciliary muscle belongs to the multiple unit class of smooth muscles

Key words accomodation – astigmatism – ciliary muscle – pilocarpine – smooth muscles

Smooth muscles differ greatly as regards the mechanism of activation In one class activity is initiated as in skeletal muscles predominantly by motor nerve impulses This class includes the vascular and pilomotor muscles and the intra ocular smooth muscles The second class the visceral muscles those of the digestive tract the ureter and the uterus are characterized by automaticity This distinction is correlated with another difference Visceral smooth muscles behave physiologically as a syncytium intramuscular conduction with a velocity of 3 7 cm/sec is an important factor in the coordination of their activity In the first class this factor plays no role or only a minor one Each muscle seems to consist of numerous separate units which by appropriate stimulation can be made to contract in small groups or even individually (Prosser 1962)

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Table I
Maximal astigmatism after pilocarpine

Subject	Experiment	Control
LM	−1.0 × 30°	−0.25 × 60
KA	−1.0 × 0°	±0
JG	−0.75 × 0°	±0
ML	−1.0 × 0°	±0
JI	−1.5 × 0°	±0
AJ	−1.0 × 10°	±0
BP	−2.0 × 10°	−1 × 0°
KP	−0.75 × 20°	±0
BP	−0.75 × 10°	±0
AJ	−1.25 × 10°	±0

optimal concentration of pilocarpine the experiments were performed on the right eyes of 10 subjects 20 to 28 years of age. All eyes were free from measurable astigmatism, they were emmetropic or had small spherical ametropias. The refraction was measured according to Donders' method before each experiment and with 5 to 10 min intervals during the experiments.

A cotton wool pledget saturated with 10 % pilocarpine drops with 0.5 % methylcellulose (Isopto pilocarpine® 10 %) was applied to the anesthetized conjunctiva bulbi over the ciliary body at the horizontal temporal meridian for 3 min. The eyelids were held by the fingers of the examiner to avoid blinking.

The controls were performed some days later. A single drop of Isopto pilocarpine® 2 % was instilled in the lower fornix in the usual way and spread out over the conjunctiva by blinking.

In each case the pilocarpine gave miosis and a moderate amount of myopia. The resulting astigmatism is presented in Table I. The maximal astigmatism was usually achieved 15 to 20 min after the application of pilocarpine. It was seen that in every case the experiments resulted in astigmatisms with the rule of about 1 diopter which seems to mean that at least half the circumference of the ciliary muscle was influenced by the pilocarpine. In the control series only two subjects developed astigmatism.

There is no general agreement in the literature as to when the maximum effect of pilocarpine on the facility of aqueous humour outflow occurs in man Some of the discrepancies might be explained by different techniques of administration of the drug (single doses or continuous administration) different concentrations different kinds of eyes (healthy or glaucomatous) and different time intervals between the administration of the eye drops and the measurement In the reports referred to below the time interval is varied and sometimes not stated Becker & Friedenwald (1953) studied 63 glaucomatous patients In 74 eyes miotics improved facility of outflow associated with a decreased intraocular pressure but in 29 eyes the pressure was normalized without improvement of facility of outflow Krill (1964) observed an increase in facility of outflow in 16 eyes of 23 while Willets (1969) reported no significant increase in outflow facility in normal subjects Kronfeld (1964) instilled a single dose of pilocarpine in patients with glaucoma and performed tonography 4 to 6 hours later He found the drug as being very ineffective on outflow mechanism in the majority of human eyes

In other studies the estimations of the outflow facility were performed within a few hours after the administration of the drug and they all show a significant increase in the facility of outflow Thus Linner (1958) gave pilocarpine topically twice with an interval of 1 hour and performed the tonography 1 hour after the second administration in normal and glaucomatous human eyes Makabe (1970) studied glaucomatous patients 1 hour after the administration of the drug Kronfeld (1967) studied ocular hypertensives and the tonographies were performed 4 hours after instillation of the drug In a recent study Barsam (1972) observed the change in intraocular pressure and facility of outflow after one single drop of 2% pilocarpine in glaucomatous patients The maximum rise in the coefficient of facility of aqueous outflow occurred after 2 to 4 hours

The present study describes the effect of one single dose of pilocarpine on the facility of outflow on the intraocular pressure on the change in refractive state and on the pupil size in normal subjects The effects are recorded half an hour 1 2 and 4 hours after the instillation of the drug

Material and Methods

The right eye of six clinically eye healthy subjects aged between 23 and 34 years were studied

The refractive error of each eye was determined using Donders subjective method and changes in the refractive state are expressed in diopters

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THE EFFECT OF A SINGLE DOSE OF PILOCARPINE ON THE FACILITY OF AQUEOUS OUTFLOW AS ESTIMATED BY USE OF A CONSTANT PRESSURE TECHNIQUE

BY

WILLIAM THORBURN

The effect of pilocarpine on the facility of aqueous outflow in normal human eyes was investigated up to 4 hours after the instillation of a 4% solution The intraocular pressure the size of the pupil and the change in refractive state were also recorded The present results indicate that the maximum increase in the facility of outflow occurs a half to one hour after the instillation of the drug which coincides with that of the change in refractive state

Key words constant pressure technique – facility of outflow – force recording – intraocular pressure – pilocarpine – pupil size – refractive state

Abbreviations

ΔV change in displaced volume
P_a intraocular pressure determined by repeated measurements by applanation tonometry
P_t intraocular pressure during the experiment
C_{ct} facility of outflow determined by recordings of applanation force at constant intraocular pressure
C_{ct6} C_{ct10} C_{ct} determined at a P_t of 6 and 10 mmHg respectively added to the P_a
P_v episcleral venous pressure
t time in min

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The refractive error of each eye was determined using Donders subjective method and changes in the refractive state are expressed in dioptres

The diameter of the pupil was measured with callipers and estimated with an accuracy of 0.5 mm. All measurements were performed in constant illumination.

The facility of outflow was estimated by use of a constant pressure technique based on applanation of the cornea during simultaneous estimation of the intraocular pressure (Linner & Thorburn 1971, Thorburn 1972a, 1972b). The applanating surface is a wall of a pressure chamber, the central aperture of which is covered with a flexible membrane. Any pressure difference between the eye and the pressure chamber is indicated by a curvature of the part of the membrane covering the aperture. The applanating force is regulated by means of an electric signal. The size of the signal indicates the degree of curvature of the membrane. In this way the apparatus measures and records continuously the applanating force, and at the same time keeps the intraocular pressure at a nearly constant, predetermined value, in principle equal to the pressure in the pressure chamber. The applanated area is calculated according to the Imbert-Fick law from the applanating force and the intraocular pressure. The displaced volume is calculated as if it was a spherical segment with the base area equal to the applanated area.

Briefly, the experiment was performed as follows. With the subject sitting, the intraocular pressure was measured repeatedly by applanation tonometry. The measurements were continued for about 10 min, until there was no further essential change, and the stabilized reading (P_a) was accepted as the intraocular pressure on which the P_t level was based. The apparatus was then applied and the applanating force was recorded with P_t preset to $P_a + 6$ mmHg for 3 min and without interrupting the recording the P_t was raised to $P_a + 10$ mmHg for another 3 min. From the recording, changes in the displaced volume occurring during the time of constant intraocular pressure can be observed. The continuous increase of displaced volume due to loss of aqueous humour can be calculated.

Using recordings of applanating force at constant intraocular pressure, the facility of outflow (C_{cp}), expressed in μl/mmHg/min, is calculated according to the equation

$$C_{cp} = \frac{\Delta V}{t\,(P_t - P_a)}$$

C_{cp6} and C_{cp10} represent the calculated facilities at the P_t levels 6 and 10 mmHg respectively added to the P_a. The symbol ΔV represents the difference between the displaced volumes at the start and at the end of each P_t level. No correction due to a possible change in P_a is introduced.

Procedures. On the first day, the pre-treatment values of pupil diameter, refractive state, intraocular pressure and facility of aqueous outflow were measured. On the following experimental days, the diameter of the pupil was first measured. One drop of 4 % pilocarpine hydrochloride dissolved in water was instilled in the lower conjunctival sac. The lower eyelid was pulled downwards for 1 min to avoid loss through the lacrimal drainage system. After the instillation of the drug, the change in refractive state and the pupil size were observed at half an hour, 1, 2 and 4 hour intervals. On the second day, recordings of the applanating force were made, preceded by applanation measurements of the intraocular pressure, at half an hour and 4 hour intervals. On the third day, recordings of applanating force were made at 1 hour, and on the fourth day, recordings of applanating force were made 2 hours after the instillation of

the drug The drug was always instilled at about the same time of day to avoid diurnal variations

Results

The pre treatment values and the changes induced by pilocarpine are shown in Fig 1 The increase in the coefficient of facility of outflow at both pressure levels as well as in refractive state showed their maximum at half an hour and 1 hour after the instillation of the drug These effects had almost disappeared at 4 hours The diameter of the pupil reached its minimum value at half an hour and the pupil was still markedly constricted at 4 hours There was no significant decrease in intraocular pressure at half an hour and the maximum decrease occurred at about 2 hours There was still a marked remaining decrease in intraocular pressure at 4 hours

The intra (S_w) and interindividual (S_B) variances of pupil size and change in refractive state are presented in Table I At each of the time intervals the intra and the interindividual variances of the pupil size were about equal The intraindividual variances of the change in refractive state were larger than the interindividual variances at each of the time intervals except for the one hour interval at which they were about equal

Table I

Variances within subjects (S_W) and between subjects (S_B^2) at each time interval calculated from three observations on each of six subjects

Time interval (hours)	Change in refractive state		Diameter of pupil	
	S_W	S_B^2	S_W	S_B
0			0 54	0 04
0 5	1 6	0 73	0 07	0 13
1	1 94	2 17	0 10	0 08
	0 46	0 18	0 06	0 08
4	0 07	0 00	0 13	0 28

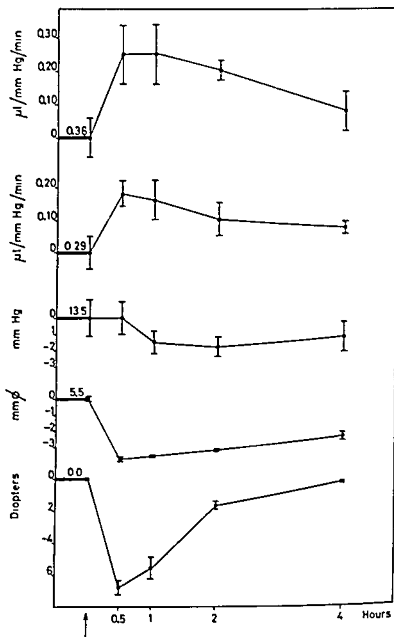


Fig. 1

Pre treatment values (heavy lines) and changes induced by instillation of 4% pilocarpine (arrow) in six normal subjects. Dots = mean values, vertical bars = standard error
From top to bottom
Facility of aqueous outflow estimated at 6 mmHg added to the intraocular pressure (C_{ep6})
Facility of aqueous outflow estimated at 10 mmHg added to the intraocular pressure (C_{ep10})
Intraocular pressure (P_a)
Diameter of the pupil
Change in refractive state

Discussion

In the interpretation of the effect of pilocarpine on the facility of outflow observed in the present study one has to consider that several factors might influence the results Observed variations might be caused by the inaccuracy of topical instillation by physiological variation and by the degree of accuracy of the technique

The present method of estimating the facility of aqueous outflow makes use of figures of displaced volume which are obtained by indirect means and by regarding the displaced volumes as spherical segments Although there is reason to believe that this is not the total volume change (Linner 1973) this was used as the results are limited to an analysis of the figures as relative values by comparing the results of the same eye as a change in the facility of outflow

The above procedure gave rise to the present results including the variation in intraocular pressure and in the facility of outflow which occurs spontaneously between different days On the other hand the possible error due to repeated tonographies is avoided

The results shown in Fig 1 indicate that the maximum effect on the facility of outflow of a single drop of pilocarpine occurs half an hour to 1 hour after the administration of the drug This coincides with the maximal increase in the refractive power and decrease in pupil size The time response of a single dose of pilocarpine on the depth of the anterior chamber and of the lens thickness in man was examined by Abramson et al (1979) They used pilocarpine 2% and found the maximum effect 45 to 60 min after the instillation The time interval for the maximum effect on the facility of outflow in the present study is not in agreement with the findings by Barsam (1972) However he used clinical routine tonography while our method is a constant pressure technique with a very moderate pressure increase in the eye

A numerical difference in the facility of outflow at the two studied levels of outflow pressure was found (Fig 1) By use of the present method a similar dependency of the facility of outflow on the outflow pressure was described (Thorburn 1978) No difference could be established between groups of different age nor between normals and those with ocular hypertension No error due to the method could be shown to induce this result There is reason to believe that the outflow facility as measured by tonography is pressure dependent but the mechanism is not clearly understood As shown in Fig 1 pilocarpine induced a similar increase in the facility of outflow at both the pressure levels studied

A decrease in intraocular pressure was not observed until 1 hour after the instillation (Fig 1) This is in accordance with previous reports by Fenton &

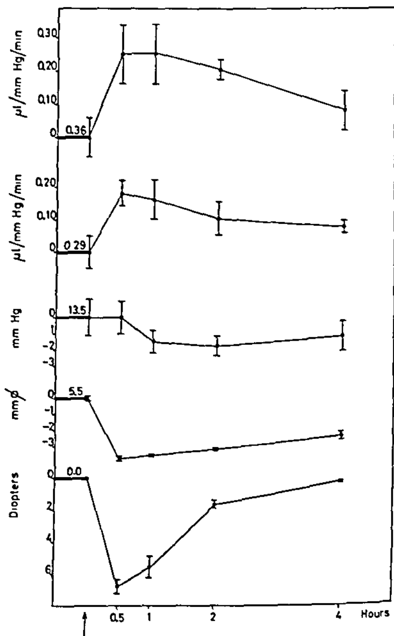


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From top to bottom
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Diameter of the pupil
Change in refractive state

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Schwartz (1963) who found a significant decrease in pressure beginning at 60 min and reaching a steady state at 75 min Drance & Nash (1971) found a maximum pressure reduction at 2 hours after the instillation of a single dose in hypertensive patients Thus there is a delay in the maximum decrease in the intraocular pressure as compared to the maximum increase in the facility of outflow The delayed change in the intraocular pressure might be due to the dilating effect of pilocarpine on the vascular bed of the anterior uvea (Alm et al 1973)

The change in the refractive state is a sensitive test on ciliary muscle contraction and the observed changes in refractive state can be assumed to reflect varying pharmacological effects The intra individual variances of refractive changes (Table I) indicate that the standardized topical instillation was far from accurate Greater accuracy is not gained by repeated experiments on one subject The pilocarpine concentration in the aqueous humour after topical administration of the drug has been determined by Asseff et al (1973) They found that lesser drug volumes gave rise to proportionately larger concentration of the drug in the aqueous humour Loss of agent through the lacrimal drainage system or over the eyelid margin was presumed to account for these discrepancies This method error is more or less involved in all clinical studies using instillation of eye drops

The estimations of the facility of outflow showed that there was no obvious increase in the variance after the pilocarpine administration as compared to that of the untreated eye This finding suggests that the error of the topical instillation did not add a significant error to the estimation of the facility of outflow Therefore the uncertainty in the facility figures will not be significantly improved by a better instillation technique

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It has recently been shown that barbiturates have effects not only on the *a* and *b* waves of the ERG but also on the *c* wave and on the standing potential (SP) of the intact sheep eye (Knave Persson & Nilsson 1973) These observations lend support to the notion that barbiturates may have dual sites of actions on the retina namely on the neuroretina and on the pigment epithelium

The aim of the present study was to establish whether this was valid also for ethyl alcohol This drug was chosen since in many of its pharmacological actions it is similar to barbiturates and its effects on the *a* and *b* waves of the ERG are well documented (Bernhard & Skoglund 1941 Bernhard Knave & Persson 1963)

Methods

The results are based on seven successful experiments made on the intact sheep eye kept in the dark adapted state The method used has recently been described in detail (Knave Møller & Persson 1972 and Knave & Persson 1973a b) It should be pointed out that with this method it is possible to record d c responses from the intact eye in long term studies under constant experimental conditions

For the ERG recordings a stimulus intensity (i e about 5 0 log units above the *b* wave threshold) was chosen so as to provide an accurate evaluation of the *a* *b* and *c* waves Duration of the stimulus light was 0 1 sec Intervals between flashes were 2 min In the records the amplitudes of the *a* and the *c* waves were measured from the isoelectric line and the amplitude of the *b* wave from the trough of the *a* wave The amplitude values were calculated from the means of two consecutive summated responses

The SP of the intact eye was recorded between a corneal electrode and an electrode placed subcutaneously at the upper bony margin of the orbit These electrodes (calomel half cells) were connected to the differential inputs of a low drift d c amplifier

The ultra short acting barbiturate (thiopental Pentothal Sodium®) and the ethyl alcohol (0% Ringer s solution) were administered through a catheter placed in a vein of the anterior leg

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A COMPARATIVE STUDY ON THE EFFECTS OF BARBITURATE AND ETHYL ALCOHOL ON RETINAL FUNCTIONS WITH SPECIAL REFERENCE TO THE C-WAVE OF THE ELECTRORETINOGRAM AND THE STANDING POTENTIAL OF THE SHEEP EYE

BY

BENGT KNAVE HANS E PERSSON and SVEN ERIK G NILSSON

The effects of barbiturate (thiopental) and ethyl alcohol on the *a* *b* and *c* waves of the conventional electroretinogram (ERG) and the standing potential (SP) of the intact sheep eye were studied and compared *I v* administration of ethyl alcohol resulted after some minutes latency in a large increase of the *c* wave amplitude A marked positive d c shift of long duration and with similar time course to that of the *c* wave increase was observed in the SP after a small negative change Barbiturate induced slow long lasting shifts of the SP with an initial negative and a subsequent positive polarity Large doses were shown to result in negative positive cyclic variations of the *c* wave For ethyl alcohol as has been shown for barbiturate the effect on the *c* wave thus appears to be linked in time to the effect on the SP No similarities whatsoever could

Key words barbiturate and ethyl alcohol – sheep electroretinography – retina – pigment epithelium

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The results are based on seven successful experiments made on the intact sheep eye kept in the dark adapted state The method used has recently been described in detail (Knave Moller & Persson 1972 and Knave & Persson 1973a b) It should be pointed out that with this method it is possible to record d c responses from the intact eye in long term studies under constant experimental conditions

For the ERG recordings a stimulus intensity (*i e* about 5 0 log units above the *b* wave threshold) was chosen so as to provide an accurate evaluation of the *a* *b* and *c* waves Duration of the stimulus light was 0 1 sec Intervals between flashes were 2 min In the records the amplitudes of the *a* and the *c* waves were measured from the isoelectric line and the amplitude of the *b* wave from the trough of the *a* wave The amplitude values were calculated from the means of two consecutive summated responses

The SP of the intact eye was recorded between a corneal electrode and an electrode placed subcutaneously at the upper bony margin of the orbit These electrodes (calomel half cells) were connected to the differential inputs of a low drift d c amplifier

The ultra short acting barbiturate (thiopental Pentothal Sodium®) and the ethyl alcohol (20% Ringer's solution) were administered through a catheter placed in a vein of the anterior leg

Results

In the experiment illustrated in Fig 1 two i v doses of ethyl alcohol (50 ml of a 20 % solution) were given (arrows). The effects on the *a* and *b* waves were in accordance with earlier findings (Bernhard, Knave & Persson 1973) i.e. small doses of alcohol reduced the *a* wave and increased the *b* wave amplitudes (Fig 1). The *c*-wave was found to increase after a latency of 2 3 min and reached an amplitude maximum after about 10 min with values six to seven times larger than the controls. Thus, in contrast to the results of the barbiturate experiments, the *b*- as well as the *c*-wave increased after injection with no preceding depression of the *c*-wave amplitude.

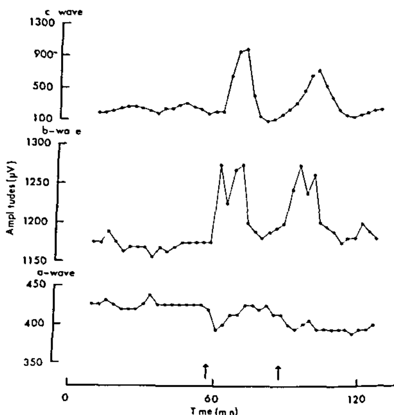


Fig 1

Effect of ethyl alcohol on *a*, *b* and *c* wave amplitudes of the dark adapted sheep eye. Two i v injections of 50 ml of a 20 % alcohol solution (Ringer) were given after 57 and 87 min respectively (arrows). Stimulus intensity 5 0 log units above *b* wave threshold. Stimulus duration 0 1 sec

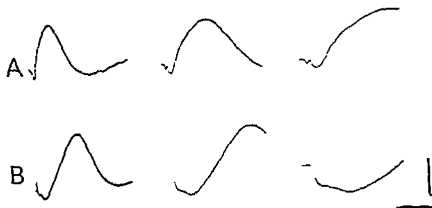


Fig 2
Effect of ethyl alcohol and thiopental on the standing potential (SP) of the dark adapted sheep eye The SP was followed 32 16 and 8 min (left middle and right recordings respectively) after i v injections of 50 ml of a 20% alcohol solution (A) and 5 mg thiopental per kg body weight (B) Time calibration 10 (right) 5 (middle) and 2.5 min (left recordings) Amplitude calibration 2 mV

In Fig 2 the effects on the SP of ethyl alcohol (upper trace A) are compared to those of barbiturate (thiopental lower trace B) In response to barbiturate an initial negative d c shift consisting of a rapid and a slow phase was followed by a slow positive shift with an amplitude maximum about 13 min after the injection (lower left trace sweep time 32 min) After alcohol the negative d c shift was very short less than 2 min It was followed by a marked positive shift with an amplitude maximum about 8 min after the injection (upper left trace sweep time 32 min) In order to demonstrate the details the initial parts of the left recordings on expanded time display are shown in the middle (sweep time 16 min) and in the right (sweep time 8 min) recordings In these recordings it can be seen that also after ethyl alcohol the negative d c shift consisted of a rapid and a slow phase However the total negative shift was much shorter in duration than with barbiturate Furthermore it is shown that the greater part of the positive d c shift after alcohol coincided in time with the slow negative d c shift after injection of barbiturate It thus seems that the changes of the SP in response to ethyl alcohol and thiopental are basically similar as to shape but markedly different as to latency

Discussion

The foregoing results show that administration of ethyl alcohol gives rise to profound alterations in the *c*-wave and the SP of the intact eye. Furthermore the effects on the *c*-wave appear to be linked in time to the effect on the SP. Since the *c*-wave and the SP are known to be generated mainly by the pigment epithelium (Noell 1954, Brown & Wiesel 1961, Gouras 1969, Steinberg, Schmidt & Brown 1970) the results support the view that ethyl alcohol influences the functions of the pigment epithelium of the retina.

The mechanism for this effect is unknown but several explanations are possible. The normal functional relationship between receptors and pigment cells (Steinberg, Schmidt & Brown 1970) may in some way be disturbed. The membrane excitability of the pigment epithelial cells may also be altered. The latter supposition is supported by the reports that ethyl alcohol influences the membrane excitability of frog muscle fibres (Knutsson 1961, Inque & Frank 1967) and of lobster axons (Houk 1969). It is evident that on the basis of the results in the present study this question cannot be settled.

It has been claimed that barbiturates influence the retina at different sites, namely on the neuroretina and on the pigment epithelial cells (Knave, Persson & Nilsson 1973). Due to the fact that no similarities were observed between the effect of alcohol on the *a*- and *b*-waves on one hand and the effect on the SP on the other, it may be hypothetized that alcohol also has a similar dual site of action on the retina.

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THE SOURCE OF LIGHT OF THE ANOMALOSCOPE WITH A NOMOGRAM TO CALCULATE THE GREEN RED QUOTIENT

BY

ULF HALLDEN

The readings of the anomaloscope are influenced by the colour temperature of the lamp The voltage across the lamp should be controlled by a voltmeter a new lamp should be allowed to burn for 24 hours before the instrument is taken into use the lamp should be discarded as soon as there is a visible darkening of the glass The precision of measurement is improved if the lamp is burned at about 75% of the rated voltage

Key words anomaloscope – green red quotient – colour temperature – deuteranomalous – protanomalous

The easiest way to detect deficiencies in the red green system of colour vision is by pseudoisochromatic plates Some cases do however remain doubtful For these cases the anomaloscope is a valuable help If it is wished not only to detect whether or not colour vision is impaired but also to determine the kind and degree of deficiency the anomaloscope is indispensable

When using the anomaloscope it is necessary to know the normal value of the Rayleigh equation The source of light of the original instrument of Nagel was a Nernst lamp The normal value was between the steps 46 to 48 on the scale of the instrument (Nagel 1907) When the maker later wanted to modify the instrument for modern incandescent lamps a weak neutral filter was placed

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in front of the red slit With this arrangement the normal value was about 60 Trendelenburg (1939) pointed out that this position of the normal value is inconvenient as it is near one end of the scale The region of colour mixture of protanomalous subjects will comprise only a few steps of the scale from 64 upwards Trendelenburg proposed that the neutral filter should be moved and placed in front of the green slit With the same source of light the normal value is about 41 In this way the normal value approaches the midpoint of the scale (36 5) and protanomalous and deuteranomalous subjects have nearly equal parts of the scale at their disposal

There are consequently three different types of the Nagel anomaloscope the original model without the neutral filter the modified one with the filter in front of the red slit and the modern model with the filter in front of the green slit It is important that the neutral filter is kept clean I have seen that a moderate amount of dust on the filter can change the normal value by 3 steps on the scale

Some difficulties with the normal value are caused by the source of light The colour temperature of incandescent lamps varies with the voltage *it changes* slowly during the lifetime of the lamp there are differences between different kinds of lamps and even individual differences between lamps of the same kind

The variations with the voltage are of special interest as they can cause relatively swift and irregular changes of the normal value of the instrument An increase in voltage causes an increase in the colour *temperature* the light contains a greater part of shortwaved radiation to achieve equal colour it is necessary to widen the red slit and to narrow the green one The result is a change of the normal value upwards on the scale The *distributors* of electrical energy don t usually allow great variations of voltage Variations of ± 5 % *do* not seriously impair the efficiency or the lifetime of incandescent *lamps* and are generally accepted During the present energy *crisis* however when *brown outs* are reported not only from underdeveloped areas but also from the great centres of civilisation such stability can not expected

The influence of the voltage on the readings has been investigated The determinations have been *performed* on two anomaloscopes *Schmidt and* Haensch Models I and II with the neutral filter in *front of* the green slit The source of light has been Luma Projection lamps 100 W 220 V Mod B The variations of voltage have *been* executed by a variable transformer and the potential across the lamp has been controlled by a voltmeter

It is known that the colour temperature *of* an incandescent lamp is an exponential function of the voltage across the lamp To simplify the mathematics it was decided to express *the* voltage as the logarithm of the *percentage* (p) of the

rated voltage of the lamps In this way a linear function was expected At each point, a series of 50 or 60 measurements was performed and the means and variances were calculated The results are given in Fig 1 As expected the

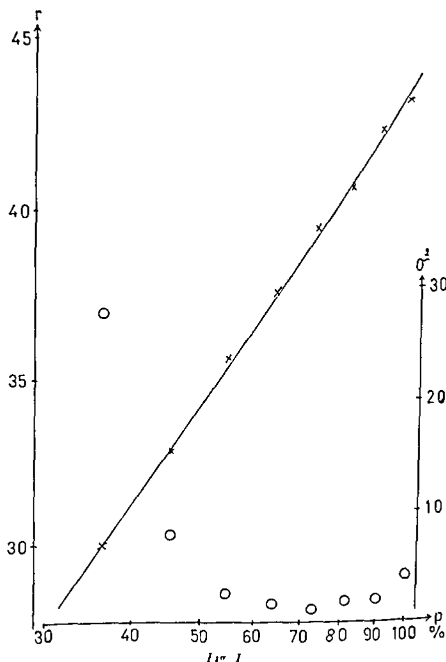


Fig 1

Abscissa voltage across the lamp in percent of the rated voltage
Ordinate to the left the mean readings of the anomaloscope (crosses)
Ordinate to the right variances (circles)

regression between lg p and the reading (r) was found to be linear The equa tion calculated from the means was

$$r = 30\,35 \ \lg p - 17\,28$$

The study of the variances gave rather surprising information The variance was lower when the voltage was a little below the rated value (The great in crease of the variance if p is below 50 is caused by the weak illumination) The variance ratio (Weatherburn 1947) was found to be statistically significant at *P* 0 01 when comparison was made between the variance at 100 % and the variances at 60 to 90 % of the rated voltage That means that the precision of measurement is improved if the lamp is burned at about 75 % of the rated voltage It might be possible that the colour temperature is less stable when the incandescent filament is very hot

It is well known that changes of the colour temperature of incandescent lamps occur at the beginning and end of the life time of the lamp It was found that the normal value of the anomaloscope increases by 1 5 to 2 0 steps of the scale during the first 24 hours of burning It then remains stable during most of the life time Near the end the values begin to decrease but at this point there is a visible darkening of the glass by precipitation of metal from the filament

Different kinds of incandescent lamps have different colour temperatures and give different readings on the anomaloscope Of importance is the differ ence between lamps of different rated voltages Most of the present experiments have been made with lamps with a rated voltage of 220 V The colour tempera ture of the corresponding 130 V lamps is higher the difference is about 4 steps on the scale

Individual differences between lamps of the same type seem to be small Since 1946 I have kept notes on the readings of 18 lamps of the same type The range of variation is about 2 steps on the scale

It is not practicable to standardize anomaloscopes so that all instruments always have the same normal value Instead the normal value is eliminated by calculating the green red quotient (grq) of v Kries (1889) This is found by the equation

$$grq \quad \frac{n\,(73\ a)}{a\,(\ 3\ n)}$$

where a is the reading of the subject and n is the normal value of the instru ment To find n use is made of the fact that the Rayleigh equation of a *normal* trichromatic observer can be *measured with* great precision and is very stable The normal observer and the subject each make an alternating series of read ings In this way variations in the source of light are *eliminated* and the mean

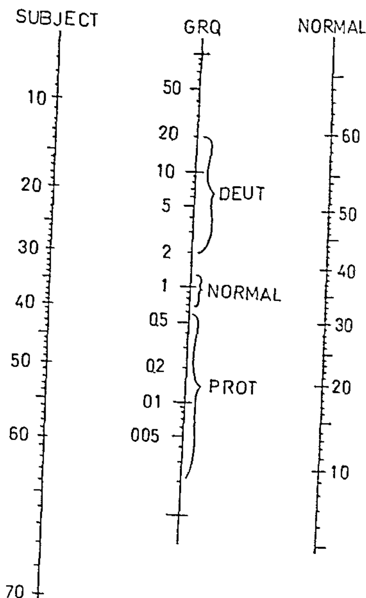


Fig 2
Nomogram to calculate the green red quotient
A straight line from the reading of the subject (left scale) to the normal value (right scale) will cross the middle scale at the grq of the subject

of the readings of the observer can be used as a measure of n To simplify the calculation of grq a nomogram is presented in Fig 2 A straight line from the reading of the subject left scale to the normal value right scale will cross the middle scale at the grq of the subject

The limits given in the nomogram between normal deuteranomalous and protanomalous should not be taken too seriously There is considerable disagreement between the authorities (Nelson 1938 Trendelenburg 1939 Sloan 1950 Schmidt 1955) and my own material of protanomalous subjects is very small

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BENIGN MUCOUS MEMBRANE PEMPHIGOID

1 Secretion of mucus and tears

BY

E BJØRN KRISTENSEN & M S NORN

Benign mucous membrane pemphigoid (BMMP) is differentiated from other bullous diseases of the skin and mucous membranes The affection is further described clinically histologically and immunologically
Twenty nine eyes affected with BMMP were subjected to quantitative measurements of the tear secretion by the tear dilution test and of the conjunctival mucus secretion by measuring the vital stained mucous thread Both secretions were found to be significantly reduced in about two thirds of the eyes (18 and 19 eyes respectively) A normal tear secretion and a normal mucus secretion were combined in no more than four eyes
Secondary rudimentary or absent Marx line was noticed in nine eyes with reduced tear secretion Epithelial cell degeneration of the tarsal conjunctiva demonstrated by vital staining with rose bengal (14 eyes) or iodonitrotetrazolium (15 eyes) was presumably secondary to reduced secretion of both tears and mucus Leucoplakia of the tarsal conjunctiva was seen in 13 eyes all with a reduced production of mucus

Key words conjunctival pemphigoid – benign mucous membrane pemphigoid – vital staining – precorneal film – Schweitzer s polygonal fluorescein pattern

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Ordinary slit lamp examination showed eight corneae to be affected (maculae blood vessel invasion dermatoid conjunctival invasion) However examinations comprising wetting time Schweitzer s polygonal pattern and vital staining yielded one or more abnormal tests in all the eyes suggesting that the cornea or at least the precorneal film is pathological in all cases of BMMP

Ever since Cooper in 1858 described a disease characterized by bullae of conjunctiva and skin with subsequent cicatricial shrinkage disagreement has prevailed concerning the name and classification of the condition This is reflected in the numerous synonyms such as pemphigus ocularis chronic cicatrizing conjunctivitis essential conjunctival shrinkage ocular pemphigoid and benign mucous membrane pemphigoid (BMMP) The disease was formerly regarded as *pemphigus* with a special localisation comprising historically a group of diseases of unknown aetiology with bullae of different sizes on apparently normal skin and/or mucous membranes Unlike in other bullous skin diseases the bulla spreading reaction is positive The same also is often true of the Nikolsky test and the prognosis is grave Histopathological studies by Civatte (1949) and in particular Lever (1953) afforded a basis for distinguishing the pemphigoids from the pemphigus group The latter group thereafter consists of *P vulgaris* *P vegetans* *P foliaceus* and *P erythematosus* characterized histologically by intra epithelial bullae and acantholysis The *pemphigoids* comprise bullous pemphigoid and BMMP where no acantholysis is seen and where the bullae are subepithelial

In recent years immunofluorescense studies have contributed towards a differentiation of the bullous diseases of the skin and mucous membranes Thus in 1964 Beutner & Jordan found the pemphigus group to contain both *circulating and in vivo bound* antibodies against the intercellular substances in stratified squamous epithelium particularly the stratum spinosum Within the pemphigoid group patients with bullous pemphigoid were found to have both circulating and in vivo bound antibodies against the basal membrane of squamous epithelium (Chorzelski et al 1968 Chorzelski & Cormane 1968 Jordan et al 19) Among patients with BMMP *on the other hand* tissue bound basal membrane antibodies have been noticed in rare cases only and circulating antibodies never (Heydenreich et al 19 2 Brody & Wuepper 1969 Bean et al 1 Hood 19 8) The antibodies in both the pemphigus group and the pemphigoid group are disease specific and belong to the IgG group In the other bullous dermatose e g erythema multiforme exudativum and chronic benign familial pemphigus neither circulating nor tissue bound antibodies have been

detected Herpetiform dermatitis constitutes an exception IgA bound on the basal membrane were demonstrated but there were no circulating antibodies (Heydenreich et al 1972)

In addition to the immunofluorescence findings the clinical picture serves as an aid in the differentiation within the pemphigoid group Thus bullous pemphigoid unlike BMMP rarely affects the eyes and there is only slight cicatrisation while the life prognosis is poor

BMMP is a rare disease occurring in one out of 15-20 000 eye patients (Bettelheim et al 1972) Its aetiology is obscure It attacks individuals aged over 60 years women about twice as often as men (Jamieson 1962) Neither a geographic nor a racial predilection has been noticed The disease runs a chronic course with periods of remission alternating with periods of exacerbation It may however apparently fade away in very old patients (Sneddon 1961) According to Hardy et al (1971) 50 % experience the first manifestations in the mouth against 30 % in the eyes and 20 % in other mucous membranes Other regions become involved after intervals of several years in most cases in the eyes and mouth (70 95 %) but also often in the nose pharynx oesophagus larynx and genitals The skin is involved in 20 50 % (Kleine Natrop & Haustein 1968) The bullae of the skin however will generally leave only superficial cicatrisation whereas the mucosal affections heal up with pronounced fibrous shrinkage a process which in rare cases may lead to fatal strictures of larynx and oesophagus (Hardy et al 1971) With this exception BMMP is as the name indicates a benign disease *quo ad vitam* whereas the prognosis for the patient s vision is much graver about one third of the affected eyes becoming blind (Hardy et al 1971 Lindemayer & Lofferer 1965) However owing to the relatively late onset of the disease in association with the slow course and often agelong remissions the stage with impaired vision will not be reached by all the patients

The first eye symptoms of BMMP are the same as those of an irritating chronic unspecific conjunctivitis with burning and smarting pain excessive tear secretion a foreign body sensation and photophobia often associated with a ropy mucous secretion which in many instances becomes mucopurulent owing to secondary infection In rare cases thin walled bullae are seen ranging in size from about 1 mm up to 1 cm These may be situated anywhere on the conjunctiva but are less frequently present on the cornea The bullae are liable to burst leaving a reddish ulceration covered by greyish macerated epithelium which when desquamated is not replaced by fresh epithelium but by fibrous cicatricial tissue (Duke Elder 1965) Each recrudescence of the disease with bullae and ulceration is followed by cicatrisation and submucous shrinkage which soon leads to entropion and trichiasis and also to progressive symblepha

ron between the palpebral and the bulbar conjunctiva This results in gradual obliteration of the fornices while the bulbar motility becomes reduced even to complete ankyloblepharon

Corneal erosions following rupture of bullae or due to trichiasis tend to develop into cloudy infiltrations or into deep undermined ulcers with associated iritis and possibly perforation leaving blurred maculae (Bettelheim et al 1972) Blood vessel invasion of the cornea is frequent forming an irregular pattern both in the cornea itself and as a pseudopterygium or pannus by invasion from the conjunctiva (Jones 1961 Duke Elder 1965)

The pronounced xerosis gradually developing owing to fibrous occlusion of the lacrimal passages constitutes an important contributary cause of the various signs and symptoms (Rycroft 1961 Ridley 1961 Bedell 1964 Bettelheim et al 1972) Localized conjunctival lesions lead to circumscribed leucoplakia like cicatrisations while the reduced tear secretion effects a universal desiccation (Ridley 1961) accentuated by the frequent presence of a certain degree of lagophthalmos with a consequent increased evaporation and continuous exposure of the eye Both the conjunctiva and the cornea will gradually become dry and finally the eye surface will have the appearance of a parchment like opaque dermatoid membrane with consequent impairment of the patient s vision (Bedell 1965 Bettelheim et al 1972 Hardy et al 1972) Rycroft (1961) in fact distinguishes between a wet and dry form of BMMP In the treatment care must be taken to keep the eye moist The surface seems however often to be hydrophobic and most patients prefer oily eye drops to aqueous (Taylor 1967) It therefore seems reasonable to suppose that other factors besides a reduced tear secretion must play a role in the development of xerosis in particular the secretion of conjunctival mucus

We therefore considered it a matter of interest to study the secretion of both tears and conjunctival mucus in eyes affected with BMMP these factors never having been estimated quantitatively Further we aimed at relating alterations of the tear and mucus secretions to conjunctival and corneal changes

Present Investigations

Material

The series studied comprised all cases of BMMP traceable within the Copenhagen region (Kommunehospitalet Rigshospitalet Frederiksberg Hospital and Finsen Institutet)

detected Herpetiform dermatitis constitutes an exception IgA bound on the basal membrane were demonstrated but there were no circulating antibodies (Heydenreich et al 1972)

In addition to the immunofluorescence findings the clinical picture serves as an aid in the differentiation within the pemphigoid group Thus bullous pemphigoid unlike BMMP, rarely affects the eyes and there is only slight cicatrisation while the life prognosis is poor

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Fig 1
Corneal macula and blood vessel invasion in a case of benign mucous membrane pemphigoid

In other words *corneal involvement in the pemphigoid process was demonstrable in no more than eight of the 29 cases*

Of these eight patients six had a reduced mucus secretion and seven had a reduced tear secretion. Five showed leucoplakia of the conjunctiva (Table 1)

Symblepharon bands The 29 eyes examined had been chosen on the basis of marked conjunctival shrinkage with a consequently diminished fornix

All these eyes also had at least one symblepharon band across the fornix (Fig 2) The majority of the bands were found in the inferior fornix passing vertically or obliquely from the inferior tarsus and adhering to the bulbar conjunctiva In three cases bands were distinctly visible in the superior fornix while in two eyes a band passed from the inferior fornix round the corneal periphery to the superior fornix

The bands ranged in number from one to four averaging two per eye Twenty three bands were situated nasally 2 temporally and 9 approximately off the middle of the lid These figures are minimum figures only distinct bands having been included in the report

In one eye two cysts were seen within the shrinkage area These were diagnosed as retention cysts and not as BMMP cysts

Leucoplakia Well defined white slightly elevated dry areas were noticed in 13 of the 29 eyes Altogether 15 patches were detected two eyes having two each All the patches were found on the inferior tarsus except one seen on the superior tarsus

A total of 29 pemphigoid affected eyes were found in 16 patients (10 females and 6 males ranging in age from 40 to 87 (mean age 71))

The criterion for being included in the series under review was an essential conjunctival shrinkage i e a marked shrinkage of the conjunctiva with a narrow fornix and with formation of vertical conjunctival folds on pulling the lid The shrinkage was not secondary to any known eye disease

Three patients with both eyes affected had only one eye included two because shrinkage was absent and one with pemphigoid whose eye could not be examined owing to total tarsorrhaphy

The eye disease was associated with bullae in the oral cavity in five patients on the genital mucosa in one and on the skin in another

Two patients were allergic (to penicillin and chloramphenicol respectively) Eight patients suffering from simple glaucoma were treated for this with *epinephrine eye drops* (14 out of 29 eyes)

Procedure

The procedure was that of vital staining with fluorescein (for estimating the wetting time) and then with a mixture of rose bengal and fluorescein (lacrimation test Norn 1972b) This was followed a few hours later by vital staining with a mixture of tetrazolium and alcian blue (Norn 1972a) The mucous thread was transferred to a slide for microscopy A sample was drawn for quantitative cytologic estimation Further a scraping and a biopsy specimen were taken and examined

At control one week later vital staining with tetrazolium and alcian blue was repeated as was also the drawing of mucous thread and cytologic samples

Results

Slit lamp examination

Ordinary slit lamp examination revealed corneal opacity in 11 of the 29 eyes Two eyes showed dermatoid conjunctival invasion over the whole cornea one eye had conjunctival invasion of the lower temporal one fourth of the cornea five had macula and blood vessel invasion (Fig 1) (two had invasion from the entire circumference and three from below and two had painting cilia) In the remaining three cases scattered minor maculae were seen which bore hardly any relation to the pemphigoid lesion



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Table I
Twenty nine pemphigoid eyes

Eye no	Mucous thread	Wetting time	Wetting time zero	Tear secretion	Schweitzer pattern	Leucoplakia	Marx line	Cornea	Rose Bengal cornea	Microfluorescein	Tetrazolium	Extra ocular disease
1 dx		+	+	+	+		+	S	+		+	
2 sin	+	+		+	+	+						
3 dx	+			+		+	+	BM	+			
4 sin	+			+		+	+	BM				
5 dx				+							+	+
6 sin		+								+	+	+
7 dx		+		+						+		
8 dx	+	+				+						+
9 sin	+	+	+		+	+		BS			+	
10 sin	+	+		+		+						
11 dx	+	+		+		+	+			+		
12 sin	+	+		+	+	+	+			+		
13 dx	+	+	+	+	+	+	+	MB	+		+	
14 sin	+	+	+	+	+	+	+	MB	+		+	
15 dx	+	+		+								
16 sin		+	+	+	+			MB	+			
17 dx	+			+		+				+		
18 dx		+		+			+			+	+	+
19 sin	+	+	+	+	+		+	SB			+	+
20 dx		+		+				M	+			
21 sin	+	+		+				M		+		
22 dx		+	+		+				+		+	+
23 sin		+									+	+
24 dx	+	+			+					+	+	+
25 sin	+	+	+					M		+	+	+
26 dx	+	+										
27 sin		+									+	
28 dx	+	+				+				+		+
29 sin	+	+				+						+

+ indicates pathological
S = skin like cornea
B = blood vessel invasion
M = macula corneae



Fig 9
Symblepharon bands in inferior fornix (pemphigoid)

The patches were oval with their longest diameter parallel with the lid margin Average length 4 1 mm (range of variation 1 10 mm) Breadth 2 3 mm (1 5 mm)

In some eyes the leucoplakia was situated close to the lid margin in others further back bordering on the inferior fornix

In two cases the leucoplakia was located laterally in five approximately off the middle of the lid and in eight medially in two of the latter across the area of the punctum lacrimale

Mucus secretion The conjunctival mucus is produced by goblet cells The mucus secretion cannot however be assessed from the number of goblet cells in a small conjunctival biopsy specimen these cells being very irregularly distributed in the conjunctiva (Kessing 1968)

The amount of mucus can be estimated in the slit lamp after vital staining with the mucus specific alcian blue An alcian blue tetrazolium mixture 0 01 ml is instilled (Norn 1972a) All normal individuals have a distinct *mucous* thread in the inferior conjunctival fornix

Altogether the 29 eyes of the present BMMP series were vital stained 55 times A normal mucous thread was found in no more than 8 % while 17 % had a minor mucous thread and 12 % a tetrazolium stained red mucous thread suggesting infection The remainder (63 %) had no mucous thread but only few and small mucous flakes in the inferior fornix

The investigation showed in other words that about two thirds had an abnormally low mucus production

The mucous thread was tranferred to a slide and the mucus area measured with a net ocular The result is shown in Table I The amount of mucus was reduced the number of vacuoles containing waste products likewise whereas the area showing red pus (neutrophils) was on an average on the large side of the normal range

The amount of mucus was significantly below the 95 % limit of the normal range (2 5 times SEM) in 19 and above in 2 of the 29 eyes

The vacoular area was reduced in 21 and increased in one of the 29 eyes

The area with neutrophilic granulocytes was increased in four cases

Each figure has been calculated as the average of usually two examinations from the same eye undertaken at an interval of one week (Table II)

The conclusion may thus be drawn *that the amount of mucus and the vacuolar area of the mucous thread in the inferior conjunctival fornix are reduced in about two thirds of affected eyes*

Tear secretion The tear secretion was estimated by Norn s lacrimal river dilution test (Norn 1965) consisting of instillation of 0 01 ml of a mixture of rose bengal and fluorescein into the inferior fornix

This gives an intensely red lacrimal river The dye becomes diluted by tears After 5 min the colour of the lacrimal river is read in the slit lamp In most normals dilution to a yellowish or a pale orange colour has been obtained by this time Only 10 % of normal eyes will still present an intensely orange colour and 0 5 % a pale red colour

Table II

The mucous thread in the inferior conjunctival fornix of patients with ocular pemphigoid and of normals measured in microscope with net ocular (mm)

	Green mucus	Red vacuoles	Red areas	Number
Pemphigoid	1 46	0 69	0 23	55
Normal	3 1 ± 0 7	1 3 ± 0 3	0 02 ± 0 16	19
Lower border of normal region	1 3	0 5	0	19

In the series under review a red or bright orange colour after 5 min must be regarded as indicative of a pathologically reduced tear secretion

The tear secretion was reduced in 18 of the 29 eyes and normal in the remaining 11

The punctum lacrimale was found to be occluded in 11 eyes in all with a resultant reduced or arrested tear secretion

Wetting time The precorneal film covers and protects the cornea The coating property of the film can be assessed by measuring the wetting time (Norn 1969) termed break up time Lemp et al (1971) and Lemp (1973)

The wetting time was studied in the following manner

One drop of fluorescein was instilled into the conjunctival sac (0 01 ml of a mixture consisting of 0 125 % fluorescein 0 3 % novesin 0 0025 % phenyl mercurinitrate and sodium chloride added to isotonicity)

The patient blinked and the precorneal film was stained evenly by fluorescein

Using a stop watch the interval was measured from the time the blinking ceased till a non stained hole occurred in the stained precorneal film

Each of the 29 BMMP affected eyes was subjected to three measurements A mean interval of 7 6 sec was arrived at

In a normal series studied by Norn a mean wetting time of 24 4 ± 3 9 sec was found for women and one of 36 4 ± 4 2 sec for men (Norn 1969) The lower 95 % limit was 14 6 sec for women and 25 9 sec for men (2 5 times SEM)

Using these criteria 84 2 % of the pemphigoid affected eyes examined had a reduced wetting time

Only four of the 29 BMMP affected eyes had normal average wetting time values (Table I) The tear secretion was reduced in all four eyes and the mucus secretion in three Three had conjunctival leucoplakia and two maculae with blood vessel invasion of the cornea

The wetting time was zero in eight cases indicating permanent presence of dry spots on the cornea Of these five had a reduced mucus secretion and five a reduced tear secretion while only one had normal secretions of both mucus and tears

Schweitzer's polygonal pattern

Fischer Schweitzer's polygonal fluorescein pattern can be studied after instillation of fluorescein (Norn 1972)

If after massage the pattern is faint or absent the test is repeated possibly after another instillation

Schweitzer's pattern was pathological in 10 of the 29 pemphigoid affected

The investigation showed, in other words, that about two thirds had an abnormally low mucus production.

The mucous thread was tranferred to a slide and the mucus area measured with a net ocular. The result is shown in Table I. The amount of mucus was reduced, the number of vacuoles containing waste products likewise, whereas the area showing red pus (neutrophils) was, on an average, on the large side of the normal range.

The amount of mucus was significantly below the 95 % limit of the normal range (2.5 times SEM) in 19 and above in 2 of the 29 eyes.

The vacuolar area was reduced in 21 and increased in one of the 29 eyes.

The area with neutrophilic granulocytes was increased in four cases.

Each figure has been calculated as the average of usually two examinations from the same eye undertaken at an interval of one week (Table II).

The conclusion may thus be drawn *that the amount of mucus and the vacuolar area of the mucous thread in the inferior conjunctival fornix are reduced in about two-thirds of affected eyes.*

Tear secretion. The tear secretion was estimated by Norn's lacrimal river dilution test (Norn 1965) consisting of instillation of 0.01 ml of a mixture of rose bengal and fluorescein into the inferior fornix.

This gives an intensely red lacrimal river. The dye becomes diluted by tears. After 5 min the colour of the lacrimal river is read in the slit lamp. In most normals dilution to a yellowish or a pale orange colour has been obtained by this time. Only 10 % of normal eyes will still present an intensely orange colour and 0.5 % a pale red colour.

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The mucous thread in the inferior conjunctival fornix of patients with ocular pemphigoid and of normals measured in microscope with net ocular (mm²).

	Green mucus	Red vacuoles	Red areas	Number
Pemphigoid	1.46	0.6[illegible]	0.[illegible]8	55
Normal	3.1 ± 0.[illegible]	1.3 ± 0.3	0.02 ± 0.16	19
Lower border of normal region	1.3	0.5	0	19

In the series under review a red or bright orange colour after 5 min must be regarded as indicative of a pathologically reduced tear secretion.

The tear secretion was reduced in 18 of the 29 eyes and normal in the remaining 11.

The punctum lacrimale was found to be occluded in 11 eyes, in all with a resultant reduced or arrested tear secretion.

Wetting time. The precorneal film covers and protects the cornea. The coating property of the film can be assessed by measuring the wetting time (Norn 1969), termed "break up time", Lemp et al. (1971) and Lemp (1973).

The wetting time was studied in the following manner.

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The patient blinked and the precorneal film was stained evenly by fluorescein.

Using a stop watch, the interval was measured from the time the blinking ceased till a non stained hole occurred in the stained precorneal film.

Each of the 29 BMMP affected eyes was subjected to three measurements. A mean interval of 7.6 sec was arrived at.

In a normal series studied by Norn a mean wetting time of 24.4 ± 3.9 sec was found for women and one of 36.4 ± 4.2 sec for men (Norn 1969). The lower 95 % limit was 14.6 sec for women and 25.9 sec for men (2.5 times SEM).

Using these criteria, 81.2 % of the pemphigoid affected eyes examined had a reduced wetting time.

Only four of the 29 BMMP affected eyes had normal average wetting time values (Table I). The tear secretion was reduced in all four eyes and the mucus secretion in three. Three had conjunctival leucoplakia and two maculae with blood vessel invasion of the cornea.

The wetting time was zero in eight cases, indicating permanent presence of dry spots on the cornea. Of these five had a reduced mucus secretion and five a reduced tear secretion, while only one had normal secretions of both mucus and tears.

Schweitzer's polygonal pattern

Fischer Schweitzer's polygonal fluorescein pattern can be studied after instillation of fluorescein (Norn 1972).

If after massage the pattern is faint or absent, the test is repeated, possibly after another instillation.

Schweitzer's pattern was pathological in 10 of the 29 pemphigoid affected

corneae In six cases the pattern was totally absent and in three partially so (nasal upper, or lower half absent) while in one only a minor defect was seen within an otherwise normal pattern

On the six eyes with a totally absent pattern three showed conjunctival invasion, while macula and blood vessel invasion were seen in the remaining three

Fluorescein vital staining

Fluorescein stains defects of the corneal epithelium the dye penetrating into the defect and further on into the intercellular spaces In normal eyes a small number of micropunctate dye elements may be seen particularly in elderly individuals and especially below and nasally

Pronounced pathological micropunctate fluorescein staining was observed in 10 of the 29 pemphigoid affected corneae The mucus secretion was reduced in seven of these 10 cases and the tear secretion in six In one both secretions were normal

Rose bengal vital staining

Rose bengal stains degenerate and dead epithelial cells The dye cannot penetrate into the intercellular spaces

In normal eyes especially of elderly individuals a few punctate elements may be found particularly below and nasally

Cornea Definitely pathological rose bengal staining was seen in seven of the 29 pemphigoid affected eyes This staining manifested itself by innumerable red dots over the whole cornea extending on to the bulbar conjunctiva especially the part of the conjunctiva that was not covered by the lid The staining was just like keratoconjunctivitis sicca

In one case it was typical with sparing of the upper non exposed corneal section

In the remaining cases however the dye was spread diffusely over the whole cornea in four cases continuing downwards over the fornix and the tarsal conjunctiva In two of the latter cases maximum staining was seen even in these areas

The tear secretion was reduced in six of the seven sicca like cases the mucus secretion only in three cases

Tarsal conjunctiva This showed maximum (grade 5) punctate staining all over in two BMMP affected eyes considerable staining in another six and weak (grade 1) in six while the remaining 15 tarsal conjunctivae remained unstained

Only weak staining at the most is seen in normal eyes

The pathological staining in eyes with BMMP was not limited to symblepharon bands or leucoplakia patches

In four of the 15 eyes with leucoplakia slight punctate staining was seen within the patch The colour however paler than that of the surrounding conjunctiva In eight leucoplakia affected eyes a ring of dots was seen round the patch

Inferior fornix was stained in the main like the tarsus

Marx line is the rose bengal stained punctate line along the lid margin of the tarsal conjunctiva which is related medially to the punctum lacrimale The line is present in all normal eyes (100 %, Norn 1972)

Marx line was absent in five of the 29 eyes while it was rudimentary and broken in four (in two eyes broken at the site of a leucoplakia patch) The line was normal in the remaining 20 eyes

The tear secretion was reduced in all nine eyes having a pathological Marx line This bears out the hypothesis that Marx line is due to the flood of tears fretting the base of the lacrimal river

Tetrazolium vital staining

Iodonitrotetrazolium stains degenerate cells with preserved enzyme activity in the corneal epithelium Normal cells very rarely become stained (Norn 1971 1972)

In the present series moderate staining was seen in 13 of the 29 corneae in most eyes extending on to the bulbar conjunctiva In six the mucus secretion was reduced and in six the tear secretion while in three both secretions were normal Pathological sicca like rose bengal staining was seen as well in four eyes only

The *tarsus* was surprisingly often stained (28 %) the superior tarsus as often as the inferior The staining of the inferior tarsus was fairly *intense* in four cases less so (grade 2) in five and minimal in six Only two eyes showed staining in a leucoplakia patch and only one eye showed staining especially on a symblepharon band In three eyes the staining extended as far as the inferior fornix

Alcian blue vital staining

Alcian blue stains specifically mucus It generally does not stain the cornea of normal eyes In rare cases however a small number of stained dots may be seen below and nasally even in normal eyes

Punctate staining of mucus was seen in 10 of the 29 pemphigoid affected

corneae In six cases the pattern was totally absent and in three *partially so* (nasal, upper, or lower half absent) while in one only a minor defect was seen within an otherwise normal pattern

On the six eyes with a totally absent pattern three showed conjunctival invasion while macula and blood vessel invasion were seen in the remaining *three*

Fluorescein vital staining

Fluorescein stains defects of the corneal epithelium the dye penetrating into the defect and further on into the intercellular spaces In normal eyes a small number of micropunctate dye elements may be seen particularly in elderly individuals and especially below and nasally

Pronounced pathological micropunctate fluorescein staining was observed in 10 of the 29 pemphigoid affected corneae The mucus secretion was reduced in seven of these 10 cases and the tear secretion in six In one both secretions were normal

Rose bengal vital staining

Rose bengal stains degenerate and dead epithelial cells The dye cannot penetrate into the intercellular spaces

In normal eyes especially of elderly individuals a few punctate elements may be found particularly below and nasally

Cornea Definitely pathological rose bengal staining was seen in seven of the 29 pemphigoid affected eyes This staining manifested itself by innumerable red dots over the whole cornea extending on to the bulbar conjunctiva especially the part of the conjunctiva that was not covered by the lid The staining was *just like keratoconjunctivitis sicca*

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battery of tests (wetting time Schweitzer s pattern vital staining) one or more disclosed pathological elements in all the eyes with BMMP This indicates a morbid condition of the cornea or at least the precorneal film in all BMMP cases which forebodes a subsequent vision threatening corneal complication A prospective investigation may possibly contribute towards clarifying which of the pathological findings constitute the poorest prognostic signs The most important pathological findings have been set out in Table I It is difficult to find any correlation between the different parameters The wetting time may be reduced in relation to an impaired tear secretion The same is true in kerato conjunctivitis sicca The wetting time is however just as often reduced in the presence of an impaired mucus secretion and may even be seen in eyes with normal secretions of both mucus and tears *This suggests the influence of other factors as well on the stability of the precorneal film* The sicca like rose bengal staining bears relation to a reduced tear secretion A normal mucus secretion cannot prevent this form of epithelial degeneration A reduced mucus secretion provokes no such sicca like rose bengal staining

The tetrazolium stainable epithelial degeneration with preserved enzyme activity seems to bear relation to reduced secretion of tears as well as of mucus Tetrazolium staining of cornea bulbar conjunctiva or tarsal conjunctiva is fairly often noticed in BMMP It discloses an epithelial degeneration differing from that stained by rose bengal Besides tetrazolium staining has been observ ed on the tarsal conjunctiva in chronic simple conjunctivitis and on the exposed parts of cornea and bulbar conjunctiva (more rarely tarsal conjunctiva) in keratoconjunctivitis sicca (Norn 1972b)

Owing to the pronounced subjective complaints and the poor prognosis of vision the requirement of treatment is almost imperative *Unfortunately how ever this* is symptomatic and as a rule ineffective In acute stages local or general corticosteroid treatment seems to be of value in some cases whereas immunosuppressive therapy e g with Azathioprin or by different forms of *irradiation* has no definite affect (Hardy et al 1971) It is important to keep the eye moist with a tear substitute (preferably of an oily nature) and by wearing a contact lens which protects the eye against drying and trichiasis and keeps the fornices open During contact lens treatment the cornea may slowly clear up the vascularisation decrease the vision improve and the patient feel more comfortable (Gasset & Kaufman 1971 Ridley 1961 Taylor 1967) Vascularized corneal tissue can in some instances be fairly easily removed by superficial keratectomy but surgical treatment should be limited to a minimum and performed exclusively in a quiescent phase (Rycroft 1961) Intervention against trichiasis is important however Further synechiolysis and fornix re construction with a view to fitting a contact lens may be of value

corneae In most cases only a few dots were present like in normal eyes Similar staining was observed on the tarsal conjunctiva

DISCUSSION

This is the first time the mucus secretion has been studied in a series of BMMP affected eyes by measuring the vital stained mucous thread in the inferior fornix and the tear secretion by the tear dilution test

We found that both the mucus secretion and the tear secretion were reduced in two thirds of the series though not necessarily at the same time Only four of the examined 29 eyes secreted normal amounts of both mucus and tears (10 had a normal mucus secretion and 11 had a normal tear secretion)

Some of the other findings may be secondary to either the reduced secretion of mucus or to that of tears

Leucoplakia was only present in eyes with a reduced mucus production

A rudimentary or totally absent Marx line was only found in eyes with a reduced tear secretion The punctum lacrimale was not occluded till after the tear secretion had been reduced for some time

The epithelial cell degeneration noticed on the tarsal conjunctiva (vital stained by rose bengal or tetrazolium) was presumably secondary to both a reduced tear secretion and a reduced mucus secretion

Mucus tends to be deposited on rough epithelial areas It is therefore only natural that a considerable amount of stained mucus may be seen on the tarsus despite a reduced mucus secretion

The mucous thread in the inferior fornix acts as a band conveyor of waste products which become deposited in vacuoles of the mucous thread The rudimentary mucous thread which in addition moves at an abnormally slow rate in pemphigoid cases (Norn 1972) accordingly constitutes a poor conveyor system The number of vacuoles is reduced

Benign mucous membrane pemphigoid (BMMP) has as the name suggests a favourable prognosis with regard to life and the skin lesions The prognosis of vision on the other hand is poor the disease leading to blindness in one third of the cases owing to corneal changes (Hardy et al 1971 Lindemayr & Lofferer 1965) In the series under review eight of the 29 corneae were more or less affected In another three cases changes were disclosed by ordinary slit lamp examination However these were hardly due to BMMP Using the stated

battery of tests (wetting time Schweitzer s pattern vital staining) one or more disclosed pathological elements in all the eyes with BMMP This indicates a morbid condition of the cornea or at least the precorneal film in all BMMP cases which forebodes a subsequent vision threatening corneal complication A prospective investigation may possibly contribute towards clarifying which of the pathological findings constitute the poorest prognostic signs The most important pathological findings have been set out in Table I It is difficult to find any correlation between the different parameters The wetting time may be reduced in relation to an impaired tear secretion The same is true in kerato conjunctivitis sicca The wetting time is however just as often reduced in the presence of an impaired mucus secretion and may even be seen in eyes with normal secretions of both mucus and tears *This suggests the influence of other factors as well on the stability of the precorneal film* The sicca like rose bengal staining bears relation to a reduced tear secretion A normal mucus secretion cannot prevent this form of epithelial degeneration A reduced mucus secretion provokes no such sicca like rose bengal staining

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The fact that no less than 14 of the 29 eyes had had epinephrine drops instilled for some length of time is thought provoking It can be asked whether epinephrine may contribute towards activating the development of BMMP The series under review is too small to throw any light on this question The epinephrine treated patients did not differ clinically from the others with pemphigoid this group also including severe cases with corneal complications

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BENIGN MUCOUS MEMBRANE PEMPHIGOID

II Cytology

BY

M S NORN and E BJORN KRISTENSEN

Patients with ocular benign mucous membrane pemphigoid have had their conjunctival epithelium examined by means of pipette samples drawn from the conjunctival fluid for estimating the cell contents and smears from the conjunctiva

Of the 29 eyes examined 24 showed metaplasia changing the columnar epithelium of a normal conjunctiva into squamous epithelium with parakeratosis in 22 of these eyes This result was achieved by analyzing three pipette samples and three smears from each eye (Out of 88 pipette samples and 92 smears 54 and 86 respectively showed abnormal squamous epithelium and in addition 32 and 48 respectively showed parakeratosis)

Neutrophilia was noticed in a scant half of the pipette samples an observation which bears out the hypothesis that the normal regeneration system has been destroyed in the presence of pemphigoid The amounts secreted of both tears and mucus are reduced The mucous thread in the inferior conjunctival fornix is rudimentary

Key words conjunctiva – ocular benign mucous membrane pemphigoid mucosal shrinkage cytology

In normal eyes the conjunctiva is covered by a non keratinized stratified epithelium Where it covers the tarsus and the fornix this epithelium is of the columnar type while the eyeball and a narrow zone along the lid margin are covered by squamous epithelium

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Benign mucous membrane pemphigoid (BMMP) has no specific histological characteristics Lever (1953) showed that the bullae are subepithelial and that there is no acantholysis BMMP thus differs from pemphigus which is characterized by intra epithelial bullae and the frequent occurrence of acantholysis In BMMP the epithelium becomes detached in its entirety including the basal membrane PAS staining may show the latter to be the separation zone (Mc Carthy 1972) Subepithelially we may see at the early stages an oedematous richly vascularized granulation tissue presenting infiltration of mainly lymphocytes and plasma cells a smaller number of neutrophilic granulocytes and in rare cases eosinophilic granulocytes (Jensen 1967) This diffuse unspecific inflammation mainly of a chronic character is much more pronounced than in pemphigus (Werf 1966) In the submucosal conjunctival stroma pronounced fibrosis is also noticed which entails cicatricial shrinkage (Andersen 1959 Taylor 1967) At the same time changes occur in the epithelium which either becomes atrophic with only few cell layers (McCarthy 1972) or irregularly thickened Associated epidermoid changes are frequently present manifesting themselves by a pronounced stratum granulosum and in some instances keratinisation Further the basal membrane will often be destroyed and have disappeared (Taylor 1967) These changes are diffuse but Jamieson (1961) has often detected circumscribed leucoplakia as well in BMMP

Similar changes may be found in the cornea Such changes are epithelial detachment from Bowman s membrane in some cases formation of bullae and ulceration and further invasion of vascularized granulation tissue with the same cellular infiltration Subsequently the thickened epithelium grows horny while Bowman s membrane becomes destroyed and the subjacent stroma becomes fibrous (Duke Elder 1965 Taylor 1967)

The object of the present study has been to examine the epithelium in BMMP *affected* eyes The investigation was based on pipette samples drawn from the conjunctival fluid for estimating the cell contents and scrapings from the conjunctiva In a future paper a report will be given on biopsies from all the conjunctival layers (The pemphigoid research group)

Present Investigations

Material

The series under review comprised 16 patients with 29 BMMP affected eyes The material is *identical* with that of a previously published investigation (Kristensen)

All the patients showed marked shrinkage of the conjunctiva causing drawing of the lid giving rise to vertical folds The shrinkage was not secondary to any known eye lesion In addition several eyes presented conjunctival growth on to the cornea and others also had leucoplakia of the conjunctiva

Eight patients had glaucoma simplex treated with epinephrine eye drops

Method

Each eye was examined using quantitative pipette samples from the inferior fornix drawn partly from the site of a symblepharon band or a leucoplakia affected area and partly from an apparently normal conjunctiva The examination was repeated one week later in connection with control of conjunctival biopsy Three pipette samples were drawn from each eye

Conjunctival scrapings were obtained in a similar manner from a morbid area and from an area outside this in the inferior fornix before and one week after biopsy three scrapings from each eye

Quantitative pipette method

The cell contents of the conjunctival fluid were analyzed by the quantitative pipette method (Norn 1960)

Interpretation of a quantitative pipette sample Pipette samples contain epithelial cells and emigrated cells The latter may be neutrophilic granulocytes eosinophilic granulocytes or lymphocytes

In a sample from a normal conjunctiva we find less than 100 neutrophils less than 100 lymphocytes no eosinophils and less than 50 nuclear squamous cells

The presence of more than 100 neutrophils (neutrophilia) indicates bacterial infection more than 100 lymphocytes (lymphocytosis) viral infection and eosinophils an allergy

The presence of more than 50 nuclear squamous cells in a pipette sample from the inferior fornix may be due to the conversion of the normal columnar epithelium into a squamous epithelium If the increased number of nuclear squamous cells occurs in association with neutrophilia or lymphocytosis this finding may however be due to the pronounced cell emigration and be secondary to this

The presence of more than 50 keratinized nuclear squamous cells is a pathological phenomenon independent of the possible existence of concurrent neutrophilia or lymphocytosis This phenomenon was found in not more than 1 per cent of a clinical material comprising 306 eyes (Norn 1960) All three eyes

displayed ectropion The finding must be interpreted as parakeratinisation of the conjunctiva

Conjunctival smears

After instillation of 0 2 % Novesin® several scrapings were performed with a platinum spatula across the conjunctival area to be examined The scraped off material was smeared carefully over a slide The preparation was fixed immediately with Pro Fixx® spray and afterwards stained with formol fuchsin and eosin

Results

Quantitative pipette sample Neutrophilia (more than 100 neutrophilic granulocytes) was found in a scant half of the preparations (39 out of 88) Neutrophilia was noticed in 15 out of 29 eyes on the first examination and in 12 out of 16 eyes on the second examination one week after biopsy

This shows that many BMMP affected eyes are bacterially infected presumably secondarily so In one pipette sample we found a small number of eosinophils suggesting allergic reaction Lymphocytosis was demonstrated in none of the eyes

All the samples also those from normal eyes contained anuclear squamous cells These coming from the lid margin were not counted in the present material

Nuclear squamous cells were present in a pathological number (more than 50) in 69 out of 88 samples (Table I)

In 32 of these we found more than 50 keratinized nuclear squamous cells suggesting parakeratinisation of the subjacent epithelium (with concurrent neutrophilia in 21 samples and was the only pathological finding in 11)

The keratinized squamous cells are very characteristic (Fig 1) They are very large cells with a small pyknotic nucleus The cytoplasm is granular bluish often with scattered vacuoles If such cells are present in fairly large numbers in the preparation they will dominate the picture even in survey microscopy

More than 50 nuclear squamous cells without keratinisation were found in 37 samples with associated neutrophilia in 15 and with neither neutrophilia nor lymphocytosis in 22 The latter finding must be interpreted as a conversion of the normal columnar epithelium in the inferior fornix into squamous epithelium

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The presence of more than 50 keratinized nuclear squamous cells is a pathological phenomenon independent of the possible existence of concurrent neutrophilia or lymphocytosis This phenomenon was found in not more than 1 per cent of a clinical material comprising 306 eyes (Norn 1960) All three eyes

Samples from symblepharon bands and plaques differed in no respect from those drawn outside these morbid areas

Of the 29 examined BMMP affected eyes 21 showed conversion into squamous epithelium or even parakeratinisation in one or more samples while samples from three eye gave inconclusive results The remaining five eyes seemed to have a normal epithelium assessed by the quantitative pipette method

Smears The smears contained a very great number of epithelial cells in the form of flakes and scattered cells The majority were cuboid epithelial cells coming from the deeper epithelial layers Here and there nuclear squamous or columnar cells might be observed

Squamous cells seemed to predominate over columnar cells in 86 of the 92 smears suggesting conversion of normal columnar epithelium into squamous epithelium (Fig 2) In 48 smears the presence of a small number of keratinized nu lear squamous cells indicated parakeratinisation In two cases whole epithelial flakes were markedly parakeratinized

No difference was demonstrable between preparations from a clinically morbid mucous membrane (symblepharon band or leucoplakia) and preparations from the surrounding fornix (Table I) This was true with regard to both smears and pipette samples

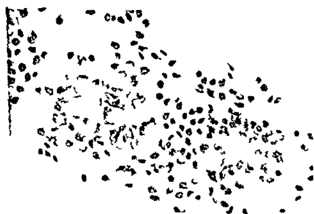


Fig 2

Smear from BMMP Squamous epithelial cells and cuboid epithelial cells

Table I

Pipette samples from the inferior conjunctival fornix of eyes with BMMP

	Symblepharon band or leucoplakia	Outside
Nuclear keratinized without neutrophilia	6	5
Nuclear keratinized with neutrophilia	12	9
Nuclear non keratinized without neutrophilia	11	11
Definitely morbid epithelium	29	25
Nuclear non keratinized with neutrophilia	8	7
Neutrophilia isolated	2	1
Others	6	10
Total	45	43

To sum up the cytologic analysis of the epithelium showed this to be definitely transformed in 54 samples (keratinized or at least converted into an abnormal squamous epithelium) and normal in 19 whereas the remaining 15 were inconclusive



Fig 1

Keratinized squamous cells in a pipette specimen Benign mucous membrane pemphigoid

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Table II

Comparison between pipette samples and smears Assessment of parakeratinized epithelium in the presence of BMMP A total of 29 eyes

Smears	Pathological	Normal
Pipette sample path	13	2
Pipette sample norm	7	[illegible]

Scrapings taken after vital staining with a mixture of tetrazolium and alcian blue showed nearly all the epithelial cells to remain unstained Only few scattered squamous cells were seen to have red granules or rods in the cytoplasm Some of these were diphtheroids while others presumably represented sparse enzyme active regions within the cytoplasm Odd epithelial cells were surrounded by green mucus or had slightly green nuclei

Comparison between pipette samples and smears Keratinisation was more easily demonstrable in a pipette sample (more than 50 large stained granular squamous cells with nucleus) than in a smear where it generally took a long time to find any definitely pathological cells

The pipette method disclosed 15 parakeratinized eyes and the smear method 20 among the total of 29 Only moderate accordance (Table II) was seen between the two types of preparations The two forms of examination supplemented each other Accordingly the overall result of analyses of three pipette samples and three smears from the 29 eyes was that conversion of normal columnar epithelium into squamous epithelium was demonstrable in 24 eyes and even parakeratinisation in 22

Discussion

In BMMP affected eyes the cell contents of the conjunctival fluid are often pathological characterized by an increased number of squamous cells and parakeratinized squamous cells Using the same pipette method such an epithelial abnormality has previously been observed in no more than 1 per cent of a clinically mixed eye material (Norn 1960) In the present material on the other hand such a condition was demonstrated in not less than one third of the preparations

Epithelial smears likewise confirmed the process of conversion into squamous epithelium or even beginning keratinisation

The preparations were taken from the inferior fornix some on a clinically abnormal mucous membrane and some on an apparently normal one Pathological findings were however equally frequent in preparations from these two areas This shows that conversion of the normal columnar epithelium into squamous epithelium occurs diffusely and that parakeratinisation likewise is diffusely present and not limited to a symblepharon band or leucoplakia patches

The former of the two analyses revealed signs of neutrophilia which suggested the presence of bacterial conjunctivitis Neutrophilia can only be demonstrated by the quantitative pipette method employed Using the smear method odd neutrophils will occasionally be found Their number depends on the sampling thus being of no decisive importance (admixture of epithelial cells depends on the depth of the scraping)

The lysozyme and other bactericides of the tears will affect the bacteria in the conjunctiva The bacteria are washed away mechanically with the tear fluid or caught by mucus from the goblet cells and carried with this down into the mucous thread in the inferior conjunctival fornix Neutrophils are attracted by bacteria emigrate into the conjunctival fluid and finally end in the mucous thread

The conditions are in many respects highly pathological in BMMP affected eyes As stated previously (Kristensen & Norn) the secretions of tears and mucus are reduced and the mucous thread rudimentary Bacteria therefore thrive better in the conjunctival sac Under these circumstances bacteria and neutrophils cannot be removed with the mucous thread which normally is milked out of the eye by blinking to end on the skin at the inner canthus *The normal regeneration system has been destroyed by the presence of pemphigoid*

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Table II

Comparison between pipette samples and smears Assessment of parakeratinized epithelium in the presence of BMMP A total of 29 eyes

Smears	Pathological	Normal
Pipette sample path	13	2
Pipette sample norm	7	7

Scrapings taken after vital staining with a mixture of tetrazolium and alcian blue showed nearly all the epithelial cells to remain unstained Only few scattered squamous cells were seen to have red granules or rods in the cytoplasm Some of these were diphtheroids while others presumably represented sparse enzyme active regions within the cytoplasm Odd epithelial cells were surrounded by green mucus or had slightly green nuclei

Comparison between pipette samples and smears Keratinisation was more easily demonstrable in a pipette sample (more than 50 large stained granular squamous cells with nucleus) than in a smear where it generally took a long time to find any definitely pathological cells

The pipette method disclosed 15 parakeratinized eyes and the smear method 20 among the total of 29 Only moderate accordance (Table II) was seen between the two types of preparations The two forms of examination supplemented each other Accordingly the overall result of analyses of three pipette samples and three smears from the 29 eyes was that conversion of normal columnar epithelium into squamous epithelium was demonstrable in 24 eyes and even parakeratinisation in 22

Discussion

In BMMP affected eyes the cell contents of the conjunctival fluid are often pathological characterized by an increased number of squamous cells and parakeratinized squamous cells Using the same pipette method such an epithelial abnormality has previously been observed in no more than 1 per cent of a clinically mixed eye material (Norn 1960) In the present material on the other hand such a condition was demonstrated in not less than one third of the preparations

Epithelial smears likewise confirmed the process of conversion into squamous epithelium or even beginning keratinisation

The preparations were taken from the inferior fornix some on a clinically abnormal mucous membrane and some on an apparently normal one Pathological findings were however equally frequent in preparations from these two areas This shows that conversion of the normal columnar epithelium into squamous epithelium occurs diffusely and that parakeratinisation likewise is diffu ely present and not limited to a symblepharon band or leucoplakia patches

The former of the two analyses revealed signs of neutrophilia which suggested the presence of bacterial conjunctivitis Neutrophilia can only be demonstrated by the quantitative pipette method employed Using the smear method odd neutrophils will occasionally be found Their number depends on the sampling thus being of no decisive importance (admixture of epithelial cells depends on the depth of the scraping)

The lysozyme and other bactericides of the tears will affect the bacteria in the conjunctiva The bacteria are washed away mechanically with the tear fluid or caught by mucus from the goblet cells and carried with this down into the mucous thread in the inferior conjunctival fornix Neutrophils are attracted by bacteria emigrate into the conjunctival fluid and finally end in the mucous thread

The conditions are in many respects highly pathological in BMMP affected eyes As stated previously (Kristensen & Norn) the secretions of tears and mucus are reduced and the mucous thread rudimentary Bacteria therefore thrive better in the conjunctival sac Under these circumstances bacteria and neutrophils cannot be removed with the mucous thread which normally is milked out of the eye by blinking to end on the skin at the inner canthus *The normal regeneration system has been destroyed by the presence of pemphigoid*

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DOUBLE FLASH HUMAN ELECTRORETINOGRAM WITH SPECIAL REFERENCE TO THE OSCILLATORY POTENTIALS AND THE EARLY PHASE OF DARK ADAPTATION
A NORMATIVE STUDY

BY

MAGNUS GJÖTTERBERG

The ERGs of 20 healthy humans were studied with a double flash technique. The interval range between flashes was 0.3–180 sec and enabled the study of photopic as well as scotopic responses. The behaviour of the a- and b-waves was compared with that of the oscillatory potentials (o.p.) during the early phase of dark adaption. The amplitude of the a- and b-waves was decreasing the shorter the interval between flashes while the amplitude of the o.p. was maximal at the 30 sec interval. The first oscillatory peak behaved differently from the others.
The recovery of the ERG from suppression by flashes was calculated for the a- and b-waves and a linear regression line was obtained. The o.p. behaved differently. Comparison with the dark adaptation curve suggested the recovery of the a- and b-wave to be linked to a photochemical process and the o.p. to a neural process.

Key words: electroretinography – double flash ERG – oscillatory potentials – dark adaptation

The size and shape of the electroretinogram (ERG) are dependent on the prevailing level of retinal dark or light adaptation when the light stimulus is delivered. One way of creating different levels of retinal adaptation is by using

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double flashes of light with varying intervals between the two flashes. N... (1957) found that when two short flashes of sufficient intensity were d... to the dark adapted retina the first flash resulted in a deep wide a-wa... a large b wave. The second flash resulted in a response of the l... type with a small a-wave and a low b wave when the interval was b... 100 and 500 msec. At a dark interval of 2 000 msec the first and second com... plexes were practically identical.

Burian and Spivey (1959) studied the double flash ERG at short inter... (15–150 msec) by varying the intensity of stimuli of very short duratio... microsec). They found that the weaker the intensity the longer must b... interval to elicit a second response. At high intensity a second ERG was ...ed in all records with a 40 msec interval. The character of the second ERG w... always essentially photopic. The authors favoured an explanation of the obser...ed phenomena based on the dependence of the ERG on photochemical processe... referring to Dowling & Wald (1958) who found a parallelism between th... electroretinographic threshold and the rhodopsin content in the retinas of v...tamin A deficient rats. Elenius (1967) investigated the ERG of the t... colour-blind eye with the double flash technique. In the dark adapted s... the ERG of the first flash was followed by a refractory period lasting f... several hundred msec. Evidently in the normal human eye in comparable con...ditions the second ERG could be related to "photopic" retinal mechanisms onl... In a later work (1969) Elenius stated that a "single flash cone ERG" could ... obtained by the second light stimulus if the dark adapted eye was stimul... by a pair of flashes of high intensity and short duration (10 msec). A peri... of complete suppression of rod activity was observed for 500 msec after th... first stimulus. Regression lines calculated for the process of recovery of rod function from suppression indicated an exponential recovery.

By using stimulus flashes of high luminance small waves, so called oscillatory potentials (o.p.), can be recorded superimposed on the b wave. Cobb & Morton (1954) described these potentials in the human electroretinogram. Studies of the o.p. have since then been made by several investigators (Bornschein & Goodman 1957, Younemura, Tsuzuki & Aoki 1962, Jacobson, Hirose & Popkin 1967, Algvere 1968, Wachtmeister 1972 and many others). The common mode of eliciting the o.p. has been to use high intensity light flashes of short duration. Heck & Rendahl (1957) used a slow flickering light and were able to record distinct oscillations. Nagata (1962) studied the photopic ERG in flicker as well as with single flashes. He noted that under photopic condition using a stimulus of shorter duration than 25 msec there was an interference between the off effect and the photopic on effect, the o.p. being overshadowed by the off effect ("h wave").

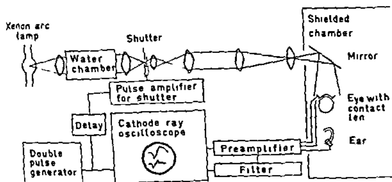


Fig 1

Block diagram of the apparatus used for eliciting the FRG A double pulse generator opened an electronically mounted shutter placed in the luminous flux of a xenon arc lamp The potentials were led off by a contact lens electrode amplified and displayed on a dual beam oscilloscope The upper sweep d splayed the a and b waves and the lower the oscillatory potentials the latter being filtered The ground electrode was attached to the ear lobe

In order to elicit distinct o p the light stimulus must be strong and the stimu lated retina must be in a favourable state of adaptation It is known that stimu lus flashes of short duration (? 5 msec) do not elicit distinct o p in dark adapta tion but in response to repetitive stimuli for example at a 30 sec interval large o p can be recorded (Algvere 1968) The optimal state of retinal adaptation can be created in different ways flicker (Heck & Rendahl 1957) background illumination (Nagata 1962) conditioning flashes (Algvere 1968) or combi nations such as slow flicker plus background illumination (Tassy 1966) condi tioning flashes plus background illumination (Wachtmeister 1972)

The purpose of the present investigation was to study the electroretinogram including the oscillatory potentials with the double flash technique The inter vals between the flashes should permit the study of scotopic as well as photopic conditions in order to register and compare the changes in behaviour of the different components of the ERG during the early phase of dark adaptation The laboratory procedure should be simple and enable the examination of an appropriate number of healthy individuals to get an estimation of the mean and standard deviation of the observations from the normal eye being the base for assessing responses obtained from diseased eyes for example with diabetic retinopathy

double flashes of light with varying intervals between the two flashes Mahneke (1957) found that when two short flashes of sufficient intensity were delivered to the dark adapted retina the first flash resulted in a deep wide a wave and a large b wave The second flash resulted in a response of the light adapted type with a small a wave and a low b wave when the interval was between 100 and 500 msec At a dark interval of 2 000 msec the first and second complexes were practically identical

Burian and Spivey (1959) studied the double flash ERG at short intervals (15–150 msec) by varying the intensity of stimuli of very short duration (10 microsec) They found that the weaker the intensity the longer must be the interval to elicit a second response At high intensity a second ERG was obtained in all records with a 40 msec interval The character of the second ERG was always essentially photopic The authors favoured an explanation of the observed phenomena based on the dependence of the ERG on photochemical processes referring to Dowling & Wald (1958) who found a parallelism between the *electroretinographic threshold and the rhodopsin content in the retinas of vitamin A deficient rats* Elenius (1967) investigated the ERG of the totally colour blind eye with the double flash technique In the dark adapted state the ERG of the first flash was followed by a refractory period lasting for several hundred msec Evidently in the normal human eye in comparable conditions the second ERG could be related to photopic retinal mechanisms only In a later work (1969) Elenius stated that a single flash cone ERG could be obtained by the second light stimulus if the dark adapted eye was stimulated by a *pair of flashes of high intensity and short duration (10 msec) A period* of complete suppression of rod activity was observed for 500 msec after the first stimulus Regression lines calculated for the process of recovery of rod function from suppression indicated an exponential recovery

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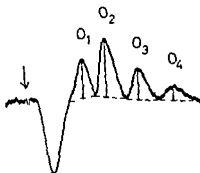


Fig 9
Method of measurement of oscillatory potentials used by Algvere & Westbeck (1979) The same method is used in the present study The height of each oscillatory peak is measured from a baseline drawn between successive troughs of the wavelets

flashes were delivered with decreasing intervals between stimulus flashes The interval between the second flash in each pair and the first one in the following pair of flashes was always 3 minutes A series of double flash ERGs was thus obtained the interval between the first and second flash being 180 100 30 10 3 1 and 0.35 sec respectively (the values were chosen to fit a logarithmic scale) In general both responses in each pair were photographed The first response in the last pair (0 35 sec) was always recorded and served as a rough check of the adaptation level In fact the b wave amplitude was about 2 % smaller in the 3 minute ERG than in the dark adapted one indicating that the initial adaptation level had not totally been regained This error was considered negligible being so small

Results

Fig 3 shows a representative series of ERG recordings from one subject The left column shows the unfiltered ERGs with the first dark adapted response at the top A large a wave typical of a high intensity stimulation was recorded as well as a b wave of large amplitude as expected in a dark adapted state The following ERGs (downwards) are the second responses with decreasing stimulus intervals With the shortest intervals typical photopic ERGs with small a and b waves were obtained The o p are distinct in these records but more easily assessed when the band pass filter was used as shown in the right column The maximal amplitude of the o p was obtained with the 30 sec interval o p could be elicited at all stimulus intervals

Material

The material comprised 20 healthy individuals with no signs of eye disease Nobody had close relatives with diabetes All subjects underwent a routine ophthalmological examination and only persons with a visual acuity of 1 0 (or more) and a normal colour sense tested by pseudoisochromatic plates were accepted Clear ocular media and a normal retinal appearance at ophthalmoscopy were ascertained Refractive errors grosser than ± 2 D were not tolerated The age range was 15 to 36 years 8 were men and 1? women One eye of each volunteer was investigated

Methods

Appara us Fig 1 shows a block diagram of the apparatus used for eliciting the ERG The light source was a xenon arc lamp the luminous flux of which was brought to the eye through a lens system The maximal luminance at the place of the eye was about 10^3 photopic candelas/m² The colour temperature of the light was about 6 000° K An electronically mounted shutter produced stimulus pulses of 25 msec duration The potentials from the eye were led off by a translucent contact lens electrode (Lawwill & Burian 1966) A small weak fixation light could be presented to the investigated eye a few seconds before the following stimulus flash The fellow eye was occluded

The signal was amplified by the aid of a preamplifier with a differential input and displayed on a dual beam cathode ray oscilloscope (Hewlett Packard 132 A) The display was photographed with a Polaroid Land camera A pulse generator capable of delivering single pulses or double pulses with different intervals opened the shutter for 25 msec thus delivering the light stimulus One cathode ray sweep displayed the a and b waves unfiltered the input being AC coupled with a lower cut off frequency of 2 Hz The other sweep displayed the oscillatory potentials using a band pass filter the 3 db points being 3 and 25 000 Hz the frequency response being flat from 80 to 5 000 Hz

Methods of measurement The amplitude of the a and b wave was evaluated by caliper square measurements The a wave was measured from the base line and the b wave from the trough of the a wave The method of measuring the b wave can be discussed measurement from the baseline would in this study cause negative values (under photopic conditions) difficult to handle In order to assess the amplitude of the o p each positive hump was measured as suggested by Algvere & Westbeck (1972) The method is shown in Fig 2

Procedures After a few drops of Mydriacyl® (Alcon lab) having been instilled in the eye the subject was placed in the dark and electrically shielded chamber for 25 minutes After good mydriasis was ascertained and surface anaesthesia was induced by Novesin® (Wander) drops the corneal contact lens was put in place under the illumination of a weak red light After another five minutes in the dark the first ERG was recorded this ERG denoted as dark adapted The next ERG was recorded after another 3 minutes thus constituting the second ERG of the first pair Then double

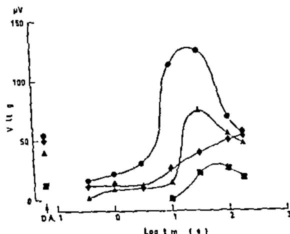


Fig 4

The mean amplitude of each oscillatory peak as recorded in the 20 subjects is plotted against log time between flashes The values in dark adaptation are shown to the left The first peak behaved differently from the others and is not regarded as belonging to the true oscillatory potentials ◆ = 1st ● 2nd ▲3rd and ■ 4th peak

that the first oscillation behaved quite differently from the others and consequently when calculating the oscillatory index by adding the values of the amplitudes the first positive hump was excluded

Fig 5 shows the amplitudes of the a and b waves and the added amplitudes of the o p plotted against log time between flashes The amplitudes in dark adaptation are shown to the left Mean and 95 % confidence limits are indicated The a and b wave amplitudes were relatively uninfluenced by the first flash at the longer intervals but declined rapidly between 10 and 3 seconds With the shortest intervals a rather stable amplitude was obtained the photopic state The oscillatory index behaved in quite a different manner the maximal response was obtained at the 30 sec interval The value was lower under more scotopic conditions and also declined in the photopic state of adaptation

The relationship log $\frac{A_1 A}{A_1}$ for the mean of the a and b waves is shown in Fig 6 A_1 is the amplitude of the response to the first flash and A to the second flash in each pair This is a way of demonstrating the decay of suppression induced by the first flash One seems justified in drawing a linear regression line for the a and b wave respectively as done in the Figure It is

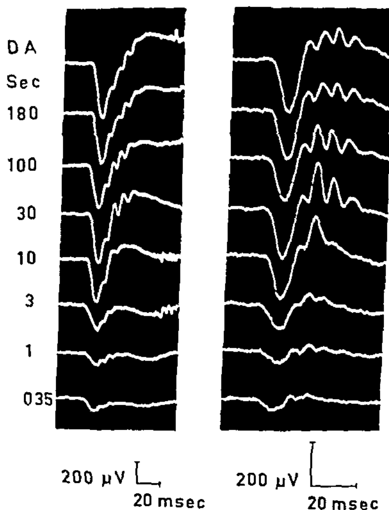


Fig 5

Series of ERG recordings from one subject The left column shows the unfiltered ERGs with the first dark adapted response at the top The following ERGs downwards are the second responses at decreasing stimulus intervals as numerals denote Large a and b waves are recorded in the dark adapted state at the short intervals photopic ERGs with small a and b waves were obtained The oscillatory potentials are distinct but more easily assessed when a band pass filter was used as shown in the right column O p were recorded at all intervals the maximal amplitudes were obtained at the 30 sec interval

In Fig 4 the mean amplitude of each oscillatory peak as recorded in the 20 subjects is plotted against log time between flashes For comparison the potentials in dark adaptation are shown to the left in the Figure (D A) It is obvious

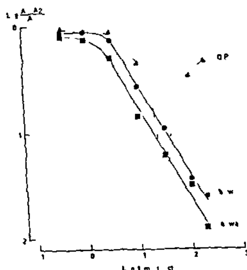


Fig 6

The relation log $\frac{A_1\ A}{A_1}$ is an expression of the suppression induced by the first flash upon the second in the pairs A_1 is the mean value of the responses to the first flash and A to the second The values are plotted against log time between flashes A linear regression line for the a and b waves can be drawn (free hand) for the intervals between 3 and 180 sec If A_1 is exchanged with $A_{m\ x}$ a similar calculation for the o p can be made The suggested curve shows quite a different appearance

Discussion

The methods of estimating and measuring the oscillatory potentials are numerous (see Fig 7) Some authors have in clinical investigations only assessed the oscillatory potentials as diminished or absent (Younemura Tsuzuki & Aoki 196? and Tassy 1966) On the other hand sophisticated procedures including Fourier analysis have been performed (Algvere & Westbeck 1972) One problem is that the rate and rise of the b wave will modify the shape and size of the oscillatory peaks In Fig 4 it was shown that the first peak behaved differently from the others more like the a and b waves In the recordings presented by Algvere & Westbeck (19 2) it can be seen that the first oscillatory peak is always present and recorded as a small hump even in response to the first flash of light in dark adaptation This observation also supports the view that

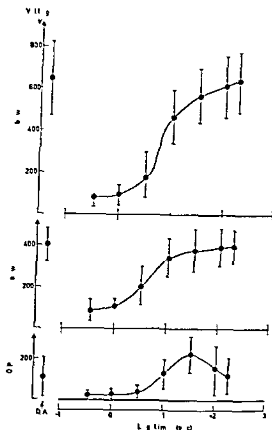


Fig 5

The mean amplitude values of the a waves b waves and o p are plotted against log time between flashes Values in dark adaptation are shown to the left 95% confidence limits are calculated according to Students t distribution (M ± 2 09 standard deviation) The amplitude of the a and b wave declined rapidly between 10 and 3 sec showing a transition from scotopic to photopic conditions The oscillatory potentials exhibited their different nature by showing a maximal amplitude at the 30 sec interval

difficult to demonstrate the behaviour of the o p in the same way but if you exchange A_I for A_{max} the values of the o p shown in Fig 6 will be obtained A_{max} for the o p is obtained at the 30 sec interval $\log \frac{A_{max}-A_o}{A_{max}}$ is then in negative infinity The regression line drawn for the oscillatory index is suggested to show the behaviour of the o p which is completely different from the a and b waves

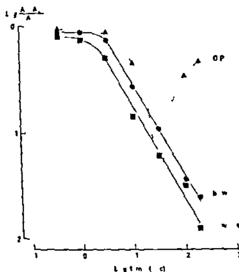


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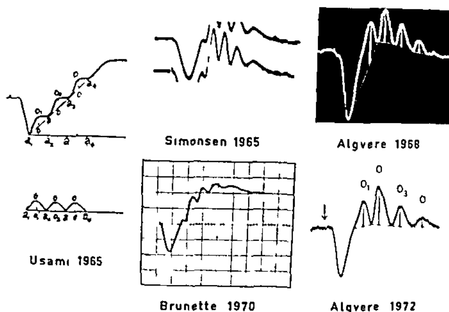


Fig 7
Different methods of measuring the oscillatory potentials Note different ways of assessing the first peak

this peak is of different nature than the following When calculating the oscillatory index by adding the amplitudes of the individual peaks it seems more correct to exclude the first peak Simonsen (1965) obviously did not include the first peak when he calculated the index but he did not explain why Brunette & Desrochers (1970) considered the o p as negative potentials and in that manner solved the problem of the first peak The different filter characteristics used complicate the comparison between different investigations as they influence the recorded amplitudes It seems reasonable to use a band pass filter which enables the o p to be recorded at a maximal amplitude at the same time as the slower potentials are cut off by a short time constant (Simonsen 1965 Algvere 1968)

It is of great interest to make the measuring procedures of the o p as uniform as possible since these components of the ERG are the subject of an increasing clinical interest as sensitive indicators of deterioration of the retinal circulation

The fast phase of recovery of the retinal sensitivity after exposure to light flashes has been attributed to neural as well as photochemical events The suppression of the amplitude of the ERG after repetitive light flashes was studied in decerebrated cats by Arden Granit & Ponte (1960) who found an exponential decay with prolonged intervals of darkness between flashes The fact that

the rate of recovery of the ERG was independent of the light period in flicker was considered to indicate that the suppression was a neural process and not a photochemically determined event The light stimuli used however were fairly weak in comparison with those in the present study Elenius (1969) found an exponential recovery of the a wave and the total ERG amplitude after an initial delay Donner & Reuter (1967) found a relationship between the inter mediate bleaching product meta rhodopsin and the logarithm of the threshold of ganglion cells of the frog retina in the course of dark adaptation The de composition of meta rhodopsin is a more rapid process than the synthesis of new rhodopsin and a desensitizing effect of meta rhodopsin will affect mainly the beginning of the dark adaptation curve Elenius raises the question whether these findings are applicable to the human eye too Rushton & Powell (1972) found in humans that following a small bleach (6 %) of rhodopsin the visual threshold was 50 times higher than expected of the bleach but the threshold soon returned to the rhodopsin level Nevertheless the experimental findings indicated that the early phase of dark adaptation was photochemical and not nervous

If the dark adaptation curve obtained after a single flash is plotted against log time the curve is linear (see Fig 8) As shown in Fig 6 the recovery of the a and b waves also is approximately linear between 3 and 180 sec if plotted against log time These results support the theory that the recovery of the ERG and the decline of the psycho physical threshold are determined by the same pro cess probably the photo chemical process postulated by Rushton & Powell (1972) Arden Granit & Ponte (1960) and Elenius (1969) found an exponen tial recovery of the ERG after light flashes In the present study however the recovery was not exponential The linear part of the regression line in Fig 6 extends from 3 to 180 sec that is about 2 log units This is in fact a much wider range than that for the exponential recovery described by Arden Granit & Ponte (0 7 log units) and Elenius (0 3 log units) It is to be noticed however that the intensity of the light stimulus was higher in the present study

In this study o p could be recorded even with the first flash after dark adaptation The second flash was able to evoke o p through the whole range of intervals even at pure photopic conditions at the 1 sec interval and most often at the 0 35 sec interval Wachtmeister (1972) studied the o p during vary ing states of retinal adaptation and found that the regeneration of the o p was facilitated at the level of retinal sensitivity where the shift from photopic to scotopic vision occurs regardless of whether this state of adaptation was induc ed by high intensity flashes adaptation to back ground illumination or created during recovery in the dark after bright pre illumination Thus the event seem ed to be mediated by a non photochemical or neural process The early phase of

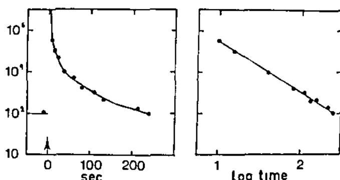


Fig 8

Dark adaptation curve obtained after a single flash Visual threshold measured in a Goldmann/Weekers adaptometer The target was viewed at a visual angle of 10° and had a maximal illumination of 6 Lux The subject indicated whether rotable black parallel stripes were vertical or horizontal The left curve shows threshold values plotted against time after flash Arrow indicates flash Initial adaptation level shown to the left The right curve is obtained if the same threshold values are plotted against log time after flash A linear curve can be drawn The rapid decline of the threshold values indicates that only a very small fraction of the rhodopsin content was bleached by the flash

dark adaptation was formerly thought to be of neural nature but Rushton & Powell (1972) recently explained it by a photochemical theory This fact does not change the opinion that the o p are produced by neural mechanisms as was suggested by Algvere (1968) The behaviour of the o p was found to be completely different from the a and b waves in agreement with the observations of other authors The a and b waves might be linked to photochemical processes but the o p reasonably cannot

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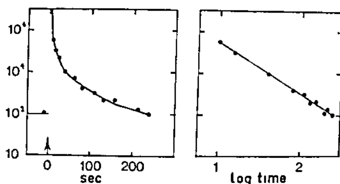


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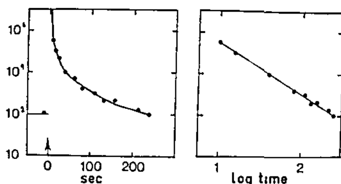


Fig 8

Dark adaptation curve obtained after a single flash Visual threshold measured in a Goldmann/Weekers adaptometer The target was viewed at a visual angle of 10° and had a maximal illumination of 6 Lux The subject indicated whether rotable black parallel stripes were vertical or horizontal The left curve shows threshold values plotted against time after flash Arrow indicates flash Initial adaptation level shown to the left The right curve is obtained if the same threshold values are plotted against log time after flash A linear curve can be drawn The rapid decline of the threshold values indicates that only a very small fraction of the rhodopsin content was bleached by the flash

dark adaptation was formerly thought to be of neural nature but Rushton & Powell (1972) recently explained it by a photochemical theory This fact does not change the opinion that the o p are produced by neural mechanisms as was suggested by Algvere (1968) The behaviour of the o p was found to be completely different from the a and b waves in agreement with the observations of other authors The a and b waves might be linked to photochemical processes but the o p reasonably cannot

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JUDICIA DE NOVIS LIBRIS

Sachsenuegcr Rudolf Augenkrankheiten im Kindesalter VEB Georg Thieme Leipzig 1973 960 pages 94 illustrations 15 tables Price DM 45 DDR

This book gives a clear presentation of childhood eye diseases As the author points out in his introduction it is difficult to differentiate between typical paediatric eye diseases and the eye diseases which appear later in life In this the author has succeeded well

The book gives an overall description of the more common complaints of the eye and its surrounding parts although for most ophthalmologists this will be common knowledge Each section is prefaced by a short introduction describing the physiological development histology and histochemistry of the area in question

However as regards child ophthalmology in particular one lacks a fuller account of the more rare eye complaints deformities and syndromes RLF (Retrolental Fibroplasia) which lately in most countries has had an unfortunate revival is treated rather cursorily Had a more through account been given of these rare diseases this book could then have been a handbook for ophthalmologists As it is it is an excellent reference book for doctors in other fields especially paediatricians and those in practice.

Literary references are plentiful but not particularly up to date The subject index is well executed

It is an attractively bound book with clear and easy to read print

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INTER EYE COMPARISON ON THE 100 HUE TEST

BY

P A ASPINALL

A test procedure is outlined and norms are given for inter eye comparisons on the 100 Hue test The norms are applied by taking the square root of the error score in each eye and obtaining the difference between the two square roots This difference is compared with values given for the 0 05 and 0 01 probability levels The calculation applies for all age groups and the norms are given for error scores less than 300 The method is particularly recommended in the clinical situation where a possible unilateral acquired dyschromatopsia can be assessed against a matched control

Key words Farnsworth Munsell 100 Hue test – acquired dyschromatopsia

The Farnsworth Munsell 100 Hue test has been widely recommended for the assessment of acquired dyschromatopsias (Verriest 1963 Lakowski 1969 Kinnear 1970 Crutzner 1972 Marre 1973) Its use in the clinical situation frequently necessitates a comparison of scores between the two eyes of a patient At present no norms exist for such comparisons although norms do exist for the total error scores of binocular tests in different age groups (Verriest 1963) Furthermore the mean score for binocular testing is not significantly different from the mean score for monocular testing in comparable age groups (Lakowski et al 1972)

The norms of Verriest indicate that the 95th percentile point or limit for a normal population ranges from an error score of 4 for subjects aged 20 to 24 years to an error score of 1 4 for subjects aged 60 to 64 years Although the

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The International Research Group on Colour Vision Deficiencies

will hold its 3rd Symposium in Amsterdam on June 25-27 1975 The main subjects will be Basic mechanisms of defective colour vision – Peripheral colour vision – Genetics of colour vision Free papers will be accepted Information can be obtained from R A Crone Dept of Ophthalmology Wilhelmina Gasthuis 1e Helmersstraat 104 Amsterdam West The Netherlands For the submission of papers (deadline 31st December 1974) a form should be requested from G Verriest Dienst Oogheelkunde Akademisch Ziekenhuis De Pintelaan 135 B 9000 Ghent Belgium

A Symposium on Immunology and Immunopathology of the Eye

will be held in Strasbourg (France) on May 20th 22nd 1974 Communications and discussions will deal with the following topics – Ocular auto immunity (retina uvea lens) – Immunopathology of cornea and conjunctiva – Viral and parasitic immunology – Immunological tests in uveitis – Immunosuppressive therapy in ophthalmology *Scientific Committee* President Prof W Boke (Kiel) Members Prof A Bronner (Strasbourg) Prof R Witmer (Zurich) Prof M H Luntz (Johannesbourg) Prof R Campinchi (Paris) Dr E Bloch Michel (Paris) Dr J P Faure (Paris) For complete information write to Prof Dr W Boke Universitats Augenklinik – Hegewischstrass 2 – 23 Kiel – West Germany

6 Left eye Box 1 (caps 1–21)
7 Right eye Box 3 (caps 43–63)
8 Left eye Box 2 (caps 22–42)

Consideration was given to the randomisation of boxes within the above framework, i.e. the toss of a coin decided whether the sequence began with right or left eye with lots drawn for the box to occupy each of the eight positions. Although this might affect the total and the partial error scores it was considered an unnecessary refinement and the above sequence was used beginning always with the right eye. Subsequent results showed no significant difference between the mean scores of right and left eyes (see Table I). This sequence has been used in the Visual Laboratory at Edinburgh for a number of years. It has also been thought necessary to replace the caps after the completion of each box in a fixed random order (Kinnear 1965, Lakowski 1969). This procedure becomes increasingly important in cases of marked visual loss. Presentation of the test in the manner described provides a stricter basis for a comparison of the scores between eyes.

Transformation of error scores

There is evidence that the frequency distribution of error scores on the 100 Hue test is positively skewed. In the norms of Verriest (1963) the total sample had a mean of 6[illegible]·1 and a standard deviation (s.d.) of 3[illegible]·6. Kinnear's (1965) population of diabetics showed a similar skewness with a mean of 123 and a s.d. of [illegible]1. In both cases the skewness was thought to be due to the nature of the measuring scale rather than to the selection of biased samples. Because of this skewness in raw error scores there is reason to suspect that the frequency distribution of the differences between right and left eye scores is itself skewed. Conversely it is probable that a transformation which normalises the scores for each eye will also normalise the frequency distribution of the difference scores. Kinnear (1970) showed that a square root transformation normalised his diabetic data. In the present study a square root, a logarithmic and a reciprocal transformation were performed on the data.

Two criteria were adopted in determining the most appropriate transformation for the inter-eye norms:

1. The frequency distribution of the difference scores should be *normal*.

The absolute level of the right and left eye scores should be independent of the size of the difference between them.

scores for both the right and left eyes of a patient may fall within the normal limit the difference in the scores between the two eyes may be sufficient to suggest that the vision of one eye is significantly poorer than the vision of the other This problem arose in a recent study of the dyschromatopsia of retinal detachment (Bhargava et al 1973) Patients with unilateral retinal detachment with macular involvement were tested on the 100 Hue test Two patients in the study had error scores of 88 and 116 for their affected eyes and 4 and 36 for their unaffected eyes respectively all scores being within the appropriate age norms Could the vision of the affected eye be considered significantly worse than the vision of the unaffected eye?

Variations in error score within the normal limits in different individuals are difficult to assess It is always possible that response criteria effects rather than variations in sensory discrimination can account for the differences in error score (Aspinall 1973) However in the case of two scores within the same individual it is reasonable to assume that factors such as motivation which affect the response criterion are constant Thus there is a firmer basis for supposing that differences in scores between eyes represent genuine visual differences It only remains to select a test procedure so that possible learning effects are randomised over both eyes It is the purpose of this paper to recommend such a test procedure and to propose norms for inter eye comparison

Procedure

The 100 Hue test was administered under standard lighting (Hubble light box illuminant C) to 113 subjects with normal vision in both eyes Thirty seven of these subjects were in age group I (under 30 years) 38 were in age group II (between 30 and 50 years) and 38 were in age group III (over 50 years)

Because of known learning and/or fatigue effects with the test it is not recommended to complete the testing on one eye before testing the second eye (Aspinall 1968) Instead the following test procedure is recommended in which a box is alternately presented to each eye (i e the right eye does the 1st 3rd 5th 7th boxes and the left eye does the 2nd 4th 6th 8th boxes no single box being presented consecutively to each eye The sequence used was as follows

1 Right eye Box 1 (caps 1–21)
2 Left eye Box 4 (caps 64–84)
3 Right eye Box 2 (caps 22–42)
4 Left eye Box 3 (caps 43–63)
5 Right eye Box 4 (caps 64–84)

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Consideration was given to the randomisation of boxes within the above framework i e the toss of a coin decided whether the sequence began with right or left eye with lots drawn for the box to occupy each of the eight positions Although this might affect the total and the partial error scores it was considered an unnecessary refinement and the above sequence was used beginning always with the right eye Subsequent results showed no significant difference between the mean scores of right and left eyes (see Table I) This sequence has been used in the Visual Laboratory at Edinburgh for a number of years It has also been thought necessary to replace the caps after the completion of each box in a fixed random order (Kinnear 1965 Lakowski 1969) This procedure becomes increasingly important in cases of marked visual loss Presentation of the test in the manner described provides a stricter basis for a comparison of the scores between eyes

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Two criteria were adopted in determining the most appropriate transformation for the inter eye norms

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The absolute level of the right and left eye scores should be independent of the size of the difference between them

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Variations in error score within the normal limits in different individuals are difficult to assess It is always possible that response criteria effects rather than variations in sensory discrimination can account for the differences in error score (Aspinall 1973) However in the case of two scores within the same individual it is reasonable to assume that factors such as motivation which affect the response criterion are constant Thus there is a firmer basis for supposing that differences in scores between eyes represent genuine visual differences It only remains to select a test procedure so that possible learning effects are randomised over both eyes It is the purpose of this paper to recommend such a test procedure and to propose norms for inter eye comparison

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4 Left eye Box 3 (caps 43–63)
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Table II

Selection of a transformation

	Age group I	Age group II	Age group III	Range (Max./Min.)
Max. (B)	110	156	188	
Min. (S)	0	12	20	168/110 = 1.53
Range	110	144	168	
$\sqrt{(B+1)}$	10.54	12.53	13.75	
$\sqrt{(S+1)}$	1.0	3.61	4.58	9.54/8.92 = 1.07
Range	9.54	8.92	9.17	
log (B + 1)	2.04	2.19	2.27	
log (S + 1)	0	1.11	1.32	2.04/0.95 = 2.15
Range	2.04	1.08	0.95	
1/(B + 1)	1.00	0.08	0.05	
1/(S + 1)	0.01	0.01	0.01	0.99/0.04 = 24.7
Range	0.99	0.07	0.04	

The smallest value of the ratio of the range indicates the appropriate transformation i.e. square root

Max. (B) represents the maximum score

Min. (S) represents the minimum score

Table III

Analysis of variance on the difference scores in the three age groups

	Difference Scores ($\sqrt{R}-\sqrt{L}$)		
	Age group I	Age group II	Age group III
Mean	0.0	−0.01	−0.1[illegible]
s.d.	1.1	1.08	1.[illegible]4
N	3	38	58

F ratio 0.[illegible]4154 (not significant)

The first criterion enables means and standard deviations to be used The second criterion results from an analysis by the present author on a small group of control subjects (see Bhargava et al 1973)

Table 1
Error scores under different transformations

		Raw Scores		Square Root		Logarithmic		Reciprocal	
		R	L	R	L	R	L	R	L
Age group I	Mean	38.0	36.6	5.83	5.77	1.45	1.47	0.07	0.04
	s d	27.2	26.3	2.25	2.07	0.42	0.32	0.16	0.03
	Max	95	110	9.80	10.54	1.98	2.04	1.00	0.11
	Min	0	8	1.0	3.0	0.0	0.95	0.01	0.01
	Kur	–0.54	0.54	–0.68	–0.61	2.02	–0.96	26.59	–0.18
	Skew	0.68	1.04	–0.01	0.46	–1.27	–0.12	5.14	1.04
Age group II	Mean	67.1	67.2	7.91	7.91	1.75	1.76	0.02	0.02
	s d	37.1	39.1	2.59	2.59	0.30	0.28	0.02	0.02
	Max	132	156	11.53	12.55	2.12	2.19	0.08	0.08
	Min	12	12	3.61	3.61	1.11	1.11	0.01	0.01
	Kur	–1.03	–0.50	–0.8	–0.86	–0.11	–0.69	2.56	2.65
	Skew	0.27	0.62	–0.19	0.14	–0.80	–0.39	1.92	1.64
Age group III	Mean	93.3	95.4	9.43	9.61	1.92	1.95	0.01	0.01
	s d	43.9	40.6	2.33	2.05	0.25	0.19	0.01	0.01
	Max	188	181	13.75	13.49	2.28	2.26	0.05	0.03
	Min	20	56	4.58	6.08	1.52	1.56	0.01	0.01
	Kur	–0.80	–0.90	–0.78	–1.07	–0.10	–1.01	4.07	0.03
	Skew	0.33	0.50	–0.09	0.23	–0.62	–0.06	1.96	0.76
Total	Mean	66.4	66.7	7.74	7.78	1.71	1.73	0.04	0.03
	s d	42.9	43.0	2.75	2.67	0.38	0.33	0.10	0.03
	Max	188	181	13.75	13.49	2.27	2.26	1.00	0.11
	Min	0	8	1.0	3.0	0	0.95	0.01	0.01
	Kur	–0.29	–0.30	–0.60	–0.77	2.91	–0.32	54.57	4.70
	Skew	0.63	0.70	–0.06	0.11	–1.35	–0.60	5.88	2.13

(s d = standard deviation Max = maximum Min = minimum Kur = kurtosis Skew = skewness R = right eye L = left eye)

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Selection of a transformation

	Age group I	Age group II	Age group III	Range (Max./Min.)
Max. (B)	110	156	188	
Min. (S)	0	12	20	168/110 = 1.53
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The smallest value of the ratio of the range indicates the appropriate transformation i.e. square root
Max. (B) represents the maximum score
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Table III
Analysis of variance on the difference scores in the three age groups

	Difference Scores ($\sqrt{R}$ $\sqrt{L}$)		
	Age group I	Age group II	Age group III
Mean	0.05	-0.01	-0.17
s.d.	1.1	1.05	1.24
N	3	38	38

F ratio 0.83154 (not significant)

Results

The results for the mean, standard deviation, maximum, minimum, kurtosis value, skewness value in each age group and for each transformation are given in Table I. For all age groups and the total population (mean = 66.4, s.d. = 42.9) the distribution of raw error scores is clearly positively skewed. The square root transformation reduces the skewness value giving a mixture of positive and negative skewness values across the age groups. The logarithmic transformation produces negatively skewed distributions in all age groups and the reciprocal transformation produces highly positively skewed distributions in all groups. Plots of the mean against the variance show that the square root transformation is the only one without a monotonic function, i.e. one which shows no systematic relation between the mean and the variance. There is no systematic relationship between either skewness and age or kurtosis and age. The figures for skewness are lowest under a square root transformation in all age groups and in the total population. The figures for kurtosis are close to zero under all but the reciprocal transformation. Confirmation that the square root transformation is the most appropriate of those selected in normalising the error scores is given in Table II (Kirk, 1968).

Before considering in detail the distribution of the difference scores an analysis of variance was carried out on the mean of the difference scores in the three age groups. [Despite the expectations discussed above, the distribution of the raw difference scores was most normal, see below. However Hartley's test showed that the variances in the three age groups were not homogeneous (Fmax = 3.4, $P < 0.01$). The transformation $(\sqrt{R}+\sqrt{I})$ produced homogeneity of variance and so was used for the significance of the mean differences.] The results are given in Table III and the analysis showed that none of the differences between the means was significant. Thus the difference scores in each age group can be considered as random samples from a larger population. This enables the data from different age groups to be added and transformations restricted to the total population.

Table IV contains the difference scores (R I) for the total population under a number of transformations. For criterion 1 the most normal distribution is formed by the difference scores themselves (column 1 of Table IV). Here the skewness value is nearest to zero and the kurtosis value is second closest to zero. The next best approximation to a normal distribution curve comes from the transformation $(\sqrt{R}+\sqrt{I})$. Here the skewness value is second closest to zero and the kurtosis value is closest to zero.

In order to satisfy criterion 2, Pearson product moment correlations were calculated between the absolute scores and the size of the difference between

Table IV

Transformations of the difference scores

	Total Population (N = 113)						
	Transformations						
	(R L)	√(R L)*	(√R √L)	log (R L)*	log (R/L)	1/(R L)	1/R 1/L
Mean	–0 [illegible]7	[illegible]2.55	–0 04	2 [illegible]0	–0 02	0 002	0 01
s d	18 03	0 41	1 16	0 0[illegible]	0 19	0 0001	0 09
Max	55	23.56	2 77	2 74	0 33	0 002	0 92
Min	–53	[illegible]1 14	–3 85	2 65	–1 11	0 001	–0 04
Kur	0 45	180 8	0 [illegible]0	–43 6	11 24	–12 8	9[illegible].5
Skew	–0 17	–1 15	–0 45	–[illegible]6 0	–2 45	0.[illegible]6	9 63

Values for √(R L) and log (R L) have been made positive by adding the constant [illegible]00 to each difference score

s d is the standard deviation
Max. is the maximum score
Min is the minimum score

Kur is the kurtosis value
Skew is the skewness value

Table V

Modulus of transformations	Age group I	Age group II	Age group III	Total
	(√R + √L)/2			
R L	0 01	–	–	0 01
√R √L	–	–	–	–
log (R L)	0 05	0 01	0 01	0 01
[illegible]	0 05	0 01	0 01	0 01
√R L	0 01	–	–	0 01
log R L	0 01	–	–	0 01
[illegible]	0 01	–	–	0 01

Correlations between the absolute scores and the size of the difference of right and left eye scores. (Dashes indicate no significant correlation; figures indicate the probability level of significance)

Results

The results for the mean standard deviation maximum minimum kurtosis value skewness value in each age group and for each transformation are given in Table I For all age groups and the total population (mean = 66 4 s d = 4? 9) the distribution of raw error scores is clearly positively skewed The square root transformation *reduces the skewness value giving a mixture of positive* and negative skewness values across the age groups The logarithmic transformation produces negatively skewed distributions in all age groups and the reciprocal transformation produces highly positively skewed distributions in all groups Plots of the mean against the variance show that the square root transformation is the *only one without a monotonic function i e one which shows no systematic* relation between the mean and the variance There is no systematic relationship between either skewness and age or kurtosis and age The figures for skewness are lowest under a square root transformation in all age groups and in the total population The figures for kurtosis are close to zero under all but the reciprocal transformation *Confirmation that the square root transformation* is the most appropriate of those selected in normalising the error scores is given in Table II (Kirk 1968)

Before considering in detail the distribution of the difference scores an analysis of variance was carried out on the mean of the difference scores in the three age groups [Despite the expectations discussed above the distribution of the raw difference scores was most normal see below However Hartley s test *showed that the variances in the three age groups were not homogeneous* (*Fmax* = 3 4 $P < 0\ 01$) The transformation ($\sqrt{R}$ $\sqrt{L}$) produced homogeneity of variance and so was used for the significance of the mean differences] The results are given in Table III and the analysis showed that none of the differences between the means was significant Thus the difference scores in each age group can be *considered as random samples from a larger population This* enables the data from different age groups to be added and transformations restricted to the total population

Table IV contains the difference scores (R L) for the total population under a number of transformations For criterion 1 the most normal distribution is *formed by* the difference scores themselves (column 1 of Table IV) Here the skewness value is nearest to zero and the kurtosis value is second closest to zero The next best *approximation to a normal distribution curve comes from* the transformation ($\sqrt{R}$ $\sqrt{L}$) Here the skewness value is second closest to zero and the kurtosis value is closest to zero

In order to satisfy criterion 2 Pearson product moment correlations were calculated between the absolute scores and the size of the difference between

The calculation is as follows

Take the square root of the right eye score i e $\sqrt{100} = 10$

Take the square root of the left eye score i e $\sqrt{49} = 7$

Find the difference between the two square roots i e $10-7 = 3$

Examine whether this difference is bigger than the limits given in Table VI (i e bigger than 2 274 at the 0 05 level or 2 993 at the 0 01 level)

If as in the present case it is then the difference in error score between eyes is outside the normal limits and the vision of the right eye is significantly poorer than the vision of the left eye

The same method applies for any age of subject and for ranges of absolute score up to approximately 200 The method may apply to higher error scores if normalisation of the absolute error scores by the square root transformation can be achieved (Kinnear s diabetic data in which the square root transformation was appropriate included error scores up to 350) In such cases both eyes would have had abnormal error scores according to the norms of Verriest (1963) but one eye would be more abnormal than the other However the present emphasis has been on scores within the normal range

A final check on the equality of the difference scores at the extreme ends of the normal range was made Fourteen subjects were selected who had an error score of less than or equal to 12 in one eye The difference scores for these subjects were compared with those for the 14 subjects from the other end of the normal range who had an error score greater than 136 in one eye. No significant difference was found between the scores ($|\sqrt{R} - \sqrt{L}|$) in the two groups (Mann Whitney U test z = 0 2) If an error score of zero should occur the constant 4 (corresponding to one inversion of caps) should be added to both error scores before taking square roots

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the scores For a measure of the absolute score $(\sqrt{R} + \sqrt{L})/2$ was used as the square root transformation normalised the raw scores (Table II) For a measure of the size of the difference in score between eyes the modulus of the difference score was used (|R L|) This was calculated for each of the transformations in Table IV The resulting significance levels of the correlation coefficients in each age group are given in Table V In order to meet criterion 2 we require a transformation which produces non significant correlations (independence) across all age groups (The transformations marked in Table V only differ from the correlations in row 1 of the Table by a change of the shape of the frequency distribution curve deviations from normality tending to reduce the value of the correlation coefficient) It is evident from Table V that only the transformation $(\sqrt{R} - \sqrt{L})$ meets the required criterion It will be recalled that this particular transformation produced one of the closest approximations to a normal distribution curve for the difference scores (Table IV) It appears therefore to satisfy both criteria and consequently to be the most appropriate transformation on which to base the norms for the difference scores

Norms

The transformation $(\sqrt{R}\ \sqrt{L})$ has a frequency distribution which satisfies criterion 1 and a modulus which when correlated with the absolute score satisfies criterion 2 If we assume that the true mean is zero (as opposed to –0 04 Table IV) the limits for normality are given in Table VI

To illustrate the use of the norms suppose the right and left eye scores are 100 and 49 respectively when the test has been administered in the recommended procedure outlined above

Table VI
Norms for inter eye comparison

Distribution $(\sqrt{R}\ \sqrt{L})$
Mean = 0 standard deviation = 1 16
At $P = 0\ 05$ the difference limit = 1 96 × 1 16 = 2 274
At $P = 0\ 01$ the difference limit = 2 58 × 1 16 = 2 993

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SPONTANEOUS HEMATOMA OF THE OPTIC CHIASMA

Report of a Case

BY

J RIISHEDE & H H SEEDORFF

A case of spontaneous hematoma in the right optic nerve and anterior part of the optic chiasm is reported Contrary to general conception it gave rise to a h mon)mous hemianopia *It is suggested that surgical exploration* of the chiasm be considered also in cases of unexplained acquired homo nymous hemianopia In the present case surgical removal of the hematoma resulted in restoration of full vision in a preoperatively practically blind eye but still a h monymous hemianopia was present

Key words neuro-ophthalmology – neurosurgery – optic chiasm – hema toma

Until recently the occurrence of spontaneous hematoma in the optic chiasma was unknown to the present authors In the literature we have been able to find only one single case which *may have been a liquified old hematoma* presenting itself as a cyst in the optic chiasma and nerve from which 2 5 cc of clear dark *amber fluid was a pirated through a needle* (Holt 1966 case 1)

Our own pat nt was a female sh p assistant who at the age of six years had had a c mpl te phthalm log cal examination wh ch disclosed *nothing abnormal* Until October 1[illegible] n w [illegible]0 years of age she had no isual compla nts and had obtained a driver s l cen e Because f unspecified vi ual fatigue ascribed to the fluorescent *lighting in the*

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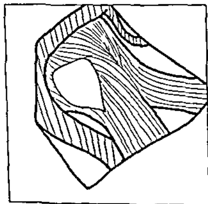
visible Following the incision of the chiasm this became flat with a shallow upper surface The vascular pattern on the chiasm and the optic nerves appeared normal Small tissue samples from three places in the wall of the cavity revealed on histological examination connective tissue glia and blood pigments but no tumor and no abnormal vessels

The day after operation the patient stated that the sight in the right eye had improved Next day she was able to read small newspaper types with the right eye alone

Thirteen days after operation she returned to her home

Ophthalmological examinations 6 and 10 days after operation Visual acuity 6/6 on both eyes Normal ophthalmoscopy Visual fields (campimetry 5/1000) Left homonymous hemianopia with small central sparing on both eyes (Fig 3)

These findings were unchanged at examinations 3 6 and 12 months after operation



2a



2b

Fig 2
Cavity of the hematoma after incission

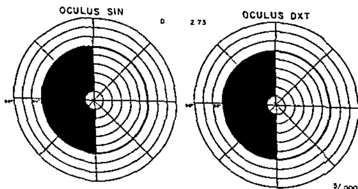
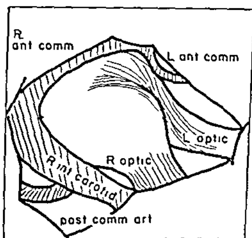


Fig 3
Visual field after the operation.

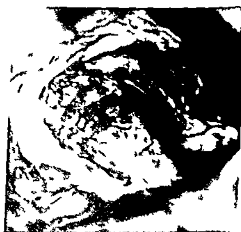
shop she was seen by an ophthalmologist who admitted her to the neurosurgical clinic. Here the visual acuity was found reduced to hand movements on the right eye, but normal on the left The right pupil reacted sluggishly to light and in a manner suggesting a nasal visual field defect Left pupil was normal as were eye movements and both eyegrounds The visual field on the right eye was difficult to determine because of the poor vision but was estimated to have a nasal hemidefect On the left eye there was a complete temporal hemianopia (5/1000) Despite these pronounced visual disturbances she stated that her practical vision was unimpaired She could read pages and the smallest letters on price marks could do her sewing and housework and drive her car as before It was only when told about the hemianopia that she admitted having formed the habit of frequently searching the entire length of the counter in the hop to avoid overlooking customers standing in front of it to her left

A full neurological examination revealed nothing abnormal X rays of the sellar region and the optic foramina were normal as was a right sided carotid angiogram A lumbar air study with planigrams of the suprasellar region showed a faint rounded shadow the size of a hazelnut in the region of the chiasm and a glioma of the right portion of the chiasma was suspected She was operated upon on December 1 19/2

The chiasm was exposed through a right sided frontal craniotomy and was almost invisible through the thickened brownish opaque arachnoid covering both the chiasm and the right internal carotid as a cyst wall When opened it contained xantochromic fluid while the CSF leaking from elsewhere was colorless The chiasm and the optic nerves were brownish yellow and the right side of the chiasm was ballooned as well as the adjoining half of the right optic nerve (Fig 1a 1b) Several areas on the swollen right half of the chiasm were black brown and appeared very thin When – under the microscope – the right margin of the chiasm was incised thick brown old blood flowed out from a cavity the size of a pea in the chiasm (Fig 2a 2b) The walls of the cavity were brownish with deposits of fibrin Its posterior wall bulged forwards like a membrane and when it was incised the normal ependyma of the third ventricle became



1a



1b

Fig 1

Optic chiama and nerves as seen from a right subfrontal approach Expansive process in right optic nerve and ant part of chiasma

human optic chiasm Nor do we have any idea about possible individual vari ations in this pattern in persons with normal visual function Therefore detailed topographical diagnosis of a lesion in the chiasm based on visual field defects and existing optic fiber maps should be made with a great deal of reservation

Traquair stated in 1942 regarding the crossing fibers in the chiasm that the close intermingling of the fasciculi is such that interference wherever situated never produces a unilateral field defect nor a pure homonymous hemianopia and homonymous hemianopia is not produced by a lateral interference with the chiasmal body This concept is still generally accepted by ophthalmologists

Our patient had a right sided hematoma in the chiasm and the adjoining part of the right optic nerve It went back to the anterior wall of the third ventricle in the midline but did *not* affect the right optic tract Nevertheless a clearcut left sided homonymous hemianopia was present postoperatively and had also existed preoperatively as far as the poor preoperative vision in the right eye permitted examination

It is probable that Traquair s statement holds true for destructive neoplastic lesions in the chiasm and for external compression of the chiasm The macro scopical appearance at the operation suggested that the hematoma had split rather than destroyed the structures and an appreciable amount of apparently intact tissue was present both in the ceiling floor and medial wall of the cavity whereas the lateral wall where the incision was made was only mem braneous A similar splitting effect is well known from intracerebral hemat omas

It is obvious that the intact crossing fibers from the nasal half of the right retina must pass through the macroscopically intact tissue The affected crossing fibers from the nasal half of the left retina may be interrupted in the hematoma or be disturbed somewhere in the macroscopically intact tissue It should namely be born in mind that a zone of disturbed function probably always surrounds even an apparently well defined and well delimited lesion and more probably so in a clinical than in an experimental case

Therefore instead of engaging ourselves in speculations about detailed fiber course we want to stress two things of clinical importance namely the existence of a spontaneous hematoma in the chiasm and the fact that this hematoma may produce a homonymous hemianopia In the present case the preoperative diagnosis was that of a tumor in the region of the chiasm This tumor however depicted only in the pneumoencephalogram proved at operation to be a closed arachnoid cyst surrounding the chiasm and not the hematoma itself This can not be expected to be revealed by any neuroradiological or other technical method

With this in mind we suggest that surgical exploration of the chiasm be

Discussion

The origin of the hematoma could not be determined The blood from the chiasm had apparently leaked out into the subarachnoid space around the chiasm resulting in a closed arachnoidal cyst appearing as a tumor shadow in the air study The patient had at the age of 8 months and at 6 years been treated for a nasal hemangioma and also at the age of six years she had had a brain operation for a tumor located in the bottom of the left lateral ventricle in the region of the foramen of Monro This tumour had given rise to increased intracranial pressure and intraventricular bleeding At the operation only small samples were removed for microscopy and the septum pellucidum was perforated to permit drainage from the left lateral ventricle through the right foramen of Monro The histological diagnosis of the tumour was Malformation with teleangiectatic angioma and the patient had a course of X ray therapy The angiogram and the air study now 14 years later showed no signs of the previous tumor There was no topographical relationship between the hematoma in the chiasm and the previous tumor site and no macro or microscopical vascular anomalies in the chiasm on the optic nerves in the tissue samples from the wall of the hematoma or in the eyegrounds

The visual fields are of particular interest The full recovery of the temporal visual field and vision in the right eye and the clearcut remaining left sided homonymous hemianopia demonstrates that lesions of the chiasm may give rise to homonymous hemianopia which is not in accordance with the general conception

Crossing of parts of the optic nerve fibers in the chiasm was first suggested by Newton in 1704 but was not generally accepted until early in the nineteenth centrury Around 1900 it was realized that the position of the nerve fibers in the visual pathways was closely related to the topography of the retina (Henschen 1893 Wilbrand Saenger 1906) and Ronne (1914) based on postmortem examination of patients gave further details of the fiber course in the chiasm and found that the crossing of the papillomacular bundle took place mainly in the posterior part of the chiasm

Animal experiments with transection of the visual pathways in monkeys have been carried out by Usher & Dean (1896) Parsons (1902) Brouver & Zeeman (1926) Polyak (1957) and others The work of Hoyt & Louis (1963) deserves special attention They made in monkeys limited retinal lesions by photocoagulation and studied the resulting nerve fiber degeneration within the optic chiasm

These studies in humans and in monkeys have not until now resulted in an indisputable and unanimous concept of the normal detailed fiber course in the

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RETINAL DETACHMENT IN THE APHAKIC EYE

BY

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In this investigation of 2 091 eyes the occurrence of retinal detachment in the aphakic eye has been studied Two groups are compared as to the incidence and type of cataract Among 187 cases of aphakic retinal detachment in the second group the relation between type of cataract type of tear cataract surgery and reattachment is recorded and the surgical procedure of the detachment discussed The significant features and the course of the aphakic detachment are outlined and the predisposition to cataractous eye is emphasized

Key words retinal detachment – aphakic – aphakic retinal detachment – retinal tear – cataract

Among the numerous functional disturbances in aphakia one of the most serious sequelae to cataract surgery is the development of a retinal detachment Not so long ago such an event usually marked the termination of the visual life of the eye (Jaffe 1972) Although the percentage of surgical reattachments of the retina has risen sharply during the last 25 years still a large number are left with poorer vision than before the detachment occurred

The purpose of this paper is to explore some of the characteristic features of the aphakic retinal detachment To present this analysis the results have been recorded in groups as demonstrated in Table I

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considered also in cases of unexplained acquired homonymous hemianopia The operation carries a minimum of risk and in the present case it resulted in restoration of full vision in a practically blind eye

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incidence of aphakic detachments originating from congenital cataracts is exactly the same in both series Since this review represents almost 2 200 cases over a span of 38 years this to some extent, contradicts the opinion that all aphakic eyes sooner or later will develop a retinal detachment (Cordes 1956 Doggart 1950) On the other hand in the adult group aphakic detachments have increased almost four times apparently demonstrating a considerable increase in the occurrence of retinal detachments among the aphakes However this finding may be explained by a comparison between the number of cataract extractions and detachment operations It appears from Table III recording the figures from our own department that the number of cataract extractions the number of detachment operations as a whole and the percentage of aphakic detachments have all increased by exactly the same amount i e three times This demonstrates quite clearly that what has really increased is not the number of aphakic detachments but the number of aphakic patients

Table II
Occurrence of aphakia and retinal detachment

	Group I 1934–1959	Group II 1961–1972
Number	900	1291
Aphakia	45 (5 [illegible]	187 (14 5 %)
Congenital cataract	15 (1 7 %)	[illegible] (1 7 %)
Adult cataract	30 (3 3 %)	165 (12 8 %)

Table III
Cataract extraction and detachment surgery

	Cataract extraction	Detachment
1940	[illegible]	51 ([illegible] %)
1960	141	54 (6 2 [illegible])
1970	14[illegible]	139 (14 4 %)

Table I
Retinal detachment in the aphakic eye

Occurrence
Type of cataract
Cataract surgery
Detachment surgery
Type of tear
Refraction before cataract surgery

The occurrence of retinal detachment in the aphakic eye may be studied either by establishing the incidence after cataract extraction or by recording the number of aphakes among cases of retinal detachment The actual incidence is difficult to assess nevertheless it is probably reasonable to estimate that 1 % to 3 % of all cataract extractions are followed by a retinal detachment This percentage may be compared to an incidence of retinal detachment in 0 01 % of all patients (Jaffe 1972 Rintelen 1962) This comparison showing that retinal detachment occurs at least 100 times more often among aphakes has led several authors (Cordes 1956 Doggart 1950 Shapland 1934) to the conclusion that a detached retina will occur sooner or later in the aphakic eye if the patient lives long enough

By recording the incidence of aphakia in any series of retinal detachment we find ranges from 5 % to 33 % (Edmund 1964 Jaffe 1972) This wide range no doubt occurs because of a tendency to refer aphakic retinal detachments to retinal centers (Norton 1963) In addition more unfavorable cases are now referred to surgery and a greater percentage of these occur among aphakes (Jaffe 1972)

The incidence of aphakia among retinal detachments in the Eye Department of Rigshospitalet is shown in Table II in which two series have been recorded In the first group up to 1959 aphakia was found in 45 cases out of 900 i e 5 % In the second group including the last 11 years 187 cases were recorded among 1 291 which amounts to 14 5 % definitely a remarkable increase the latter figure being three times as high as the former In the Table the aphakes have been divided into two groups according to whether their origin derives from a congenital or adult cataract Somewhat surprisingly it appears that the

Table V

Type of cataract surgery and reattachment

	Number	Reattached
Needling	36	20 55 %
Intracapsular	135	85 63 %
Extracapsular	14	7 50 %
No operation	2	0
Total	187	112

worsened by early subclinical detachments being present before cataract surgery but unrecognized until the opaque lens is removed (Jaffe 1972)

All recordings of the relation between type of cataract surgery detachment and reattachment unanimously fail to show any difference in the occurrence of retinal detachment after intracapsular or extracapsular extraction (Jaffe 1972 Malbran & Dodds 1964 Norton 1963) and the present series does not differ in this respect The relation between type of cataract surgery and reattachment rate is shown in Table V The number of extracapsular extractions in this series is too small to make any comparisons and the very low reattachment rate is probably more a result of the difficulty in examining and treating the detachment than a consequence of the extracapsular procedure

Although there is probably some relationship between the cataract surgery and the retinal detachment especially in those cases where the detachment follows cataract surgery after a relatively short interval many retinal surgeons however consider the predisposition to detachment to be a fundamental primary factor and that cataract surgery is perhaps just a milestone along the degenerative trail (Jaffe 1972) This view is strengthened by the clinical course of patients with bilateral detachments As pointed out some years ago in a study of familial retinal detachments the detachment may follow cataract extraction in one eye whereas in the other eye it may precede cataract surgery As mentioned previously the frequency with which congenital cataract surgery has been followed by retinal detachment by some surgeons (Doggart 1950 Cordes 1956) has been related to the number of needlings This statement could not be confirmed by this series

Table IV
Type of cataract and reattachment

	Number	Reattached
Congenital	22	13 60%
Juvenile	38	23 61%
Senile	108 (107)	67 62%
Complicated	19 (18)	9 50%
Total	187	112

That a certain type of cataract is found in those eyes that later develop a retinal detachment has been emphasized by Schepens (1951) among others and our own experience confirms this observation (Edmund 1964, Edmund & Seedorff 1968). While the type of cataract has generally been related to the location of the opacity in the lens, i.e. posterior, cortical, nuclear etc. (Malbran & Dodds 1964, Rintelen 1962, Schepens & Baker 1950), in this series four groups have been established as shown in Table IV.

The primary cataracts have been divided into three subgroups related to time of onset and extraction, separated from cases of complicated cataracts. One clinical feature demarcating the congenital group from the others is the interval between cataract operation and the detachment. The elapsed time has been averaged by Cordes (1956) to 22 years, Shapland (1962) 24.6 years and François (1959) 27 years. In our own series (Edmund 1964, Edmund & Seedorff 1968) the time span extends from 5 to 35 years with an average of 20 years. Still, the rate of reattachment is amazingly uniform in the first three groups. The most surprising finding, however, is the high incidence of reattachment in the first group. Apart from the above mentioned exceptional time of onset, the detachment in the aphakic eye following congenital cataract in many ways takes a different clinical course and furthermore establishment of diagnosis and surgical procedure is often complicated by the difficulty in visualizing the fundus.

In the last group of complicated cases, referring to complication before cataract extraction, the reattachment rate is the lowest as be might expected. Besides the aggravating influence of posterior uveitis, the prognosis may be

Table VII
Refraction before cataract surgery

Type of cataract	Type of refraction		
	Emmetropia and hypermetropia	Myopia	Excessive myopia
Congenital	10 (6) 60 %	1 (1)	2 (0)
Juvenile	13 (10) 77 %	4 (3) 75 %	12 (7) 58 %
Senile	47 (34) 73 %	10 (4) 40 %	4 (4) 100 %
Complicated	11 (7) 63 %	0	2 (0)
Total	81 (57) 71 %	15 (8) 53 %	?0 (11) 5? %

The values listed in brackets ind cate the number of reattachments and the percentage the rea tachment rate

A clinical feature of considerable interest is the refractive condition of the eye before cataract extraction Data on this parameter have only been obtained from cases having their cataracts extracted in our own department and the dispersion is shown in Table VII Out of 116 eyes 35 or 30 % were found to be myopic a little less than Schepens (1951) who found 37 % and Malbran & Dodds (1964) 46 % Nevertheless the figure is considerably lower than the rate of myopia among phakic detachments which is usually indicated around 50 % As mentioned previously many retinal surgeons consider a predisposition to be the fundamental primary factor and this relatively low percentage of myopia among the aphakes seems to confirm that along with the development of the cataract a pathological state of the peripheral choroid retina or of the vitreous body occurs (Schiff Wertheimer & Sedan 194?) This is in accordance with Surdille (194) who pointed out the frequency with which equatorial degenerations occur in non myopic eyes suffering from presenile cataracts

While 30 % myopic eyes are found in the congenital and the senile groups 60 % or double as many were found among the juveniles and furthermore

The relationship between the surgical procedure of the detachment operation and rate of reattachment is indicated in Table VI. Diathermy treatment as pointed out by several other retinal surgeons (Edmund 1964, Schepens 1951, Shapland 1934) is of slight value and has not been used as the sole procedure during the last 10 years. Scleral resection as introduced by Lindner and Shapland (1934) considerably improved the rate of cure but the procedure is inadequate as the only surgical measure. As shown by Norton (1963) extensive resections combined with 360 bucklings seem to give excellent results. However the number of scleral resections in this series is too small for any conclusions to be made. Local or segmental implants have been rather disappointing. This is probably due to the fact that the detachment in aphakia is frequently greater than can be observed and further there are multiple tiny breaks in the extreme periphery which are left insufficiently treated by the local implant (Jaffe 1972, Pasino & Santori 1967, Schepens & Baker 1950, Schepens & Marden 1961). By far the best results are obtained by the encircling procedure curing almost 2/3 of the cases. It should be mentioned that all reoperations were encirclings. Finally it should be noted that in some of the later cases vitreous surgery in the form of injection of hyaluronic acid has been employed. The surgical results in aphakic retinal detachments reveal that there has been a major improvement during the last 10–20 years. Since 1960 the rate is generally above 60 % (Malbran & Dodds 1964) but varies from 60 % (Jaffe 1972) to [illegible] % (Norton 1963).

Table VI
Surgical procedure and reattachment

	1. Operation	Reoperation	Total
Diathermy	11 (1) [illegible] %	9 (5) [illegible] %	6 (55 %)
Local implant	40 (11) 27.5 %	22 (11) 50 %	[illegible]2 (55 %)
Encircling	129 (68) [illegible] %	44 (18) 41 %	[illegible] (68 %)
Scleral resect.	4 (1) [illegible] %	2 (1) 50 %	[illegible] (50 %)
Photocoag.	1 (1)	0	1
Total	185 ([illegible]) 42 %	[illegible] (35) 49 %	11[illegible] (60 %)

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Senile	47 (34) 73 %	10 (4) 40 %	4 (4) 100 %
Complicated	11 ([illegible]) 63 %	0	2 (0)
Total	81 (57) 71 %	15 (8) 53 %	20 (11) 55 %

The values listed in brackets indicate the number of reattachments and the percentage the reattachment rate

A clinical feature of considerable interest is the refractive condition of the eye before cataract extraction Data on this parameter have only been obtained from cases having their cataracts extracted in our own department and the dispersion is shown in Table VII Out of 116 eyes 35 or 30 % were found to be myopic a little less than Schepens (1951) who found 3[illegible] % and Malbran & Dodds (1964) 46 % Nevertheless the figure is considerably lower than the rate of myopia among phakic detachments which is usually indicated around 50 % As mentioned previously many retinal surgeons consider a predisposition to be the fundamental primary factor and this relatively low percentage of myopia among the aphakes seems to confirm that along with the development of the cataract a pathological state of the peripheral choroid retina or of the *vitreous* body occurs (Schiff Wertheimer & Sedan 1947) This is in accordance with Surdille (194) who pointed out the frequency with which equatorial degenerations occur *in non myopic* eyes suffering from presenile cataracts

While 30 % myopic eyes are found in the congenital and the senile groups [illegible]0 % or double as many were found among the juveniles and furthermore

The relationship between the surgical procedure of the detachment operation and rate of reattachment is indicated in Table VI. Diathermy treatment as pointed out by several other retinal surgeons (Edmund 1964, Schepens 1951, Shapland 1934) is of slight value and has not been used as the sole procedure during the last 10 years. Scleral resection as introduced by Lindner and Shapland (1934) considerably improved the rate of cure but the procedure is inadequate as the only surgical measure. As shown by Norton (1963) extensive resections combined with 360° bucklings seem to give excellent results. However the number of scleral resections in this series is too small for any conclusions to be made. Local or segmental implants have been rather disappointing. This is probably due to the fact that the detachment in aphakia is frequently greater than can be observed and further there are multiple tiny breaks in the extreme periphery which are left insufficiently treated by the local implant (Jaffe 1972, Pasino & Santori 1967, Schepens & Baker 1950, Schepens & Marden 1961). By far the best results are obtained by the encircling procedure curing almost 2/3 of the cases. It should be mentioned that all reoperations were encirclings. Finally it should be noted that in some of the later cases vitreous surgery in the form of injection of hyaluronic acid has been employed. The surgical results in aphakic retinal detachments reveal that there has been a major improvement during the last 10–20 years. Since 1960 the rate is generally above 60 % (Malbran & Dodds 1964) but varies from 60 % (Jaffe 1972) to 85 % (Norton 1963).

Table VI
Surgical procedure and reattachment

	1. Operation	Re-operation	Total
Diathermy	11 (1) 0.8 %	9 (5) 50 %	6 (55 %)
Local implant	40 (11) 27.5 %	22 (11) 50 %	2[illegible] (55 %)
Encircling	1[illegible]9 (68) 50 %	44 (18) 41 %	81 (63 %)
Scleral resect	4 (1) 25 %	[illegible] (1) 50 %	[illegible] (50 %)
Photocoag	1 (1)	0	1
Total	185 (77) 42 %	[illegible] (85) 49 %	11[illegible] (60 %)

1956) and 40 % (Pasino & Santori 1962) Norton (1963) found no break in 16 % in contrast to *Schepens* (1951) who was unable to find any tear in only 3 % of the aphakic detachments Norton (1963) explains this discrepancy as being partly due to the small size of the breaks sometimes no larger than the caliber of a vessel and partly to the *differences in interpretation* of many suspect areas some investigators classified them as tiny breaks others as degenerative areas It is interesting to notice the differences among the four groups The figure of 51 % in the congenital group is depressing but explainable by the well known difficulty of visualising the fundus because of the small distorted pupil lens remnant opacities in the vitreous and frequently nystagmus In the complicated group vitreous haze plays a role but probably cases of exudative *detachments also make their contribution* It is however remarkable that no tear could be found in one fourth of the juvenile and senile aphakics even with the use of the current techniques of indirect binocular ophthalmo copy and *biomicroscopy with the Goldmann 3 mirror contact lens* This might be due to lack of skill in the examination techniques and might further be explained by *the different interpretations* of a tear but in our opinion in the aphakic eye *with the fundamental* anatomical and physiological changes and frequent disturbances a particular type of retinal detachment with a different pathogenesis cannot be ruled out (Edmund 1964)

The significance of the encircling surgical procedure the relatively high rate *of reattachment in cases without a tear* and last but not least *as pointed out* by Norton (1963) the discovery that failure to find a retinal break in aphakic cases did not seriously influence the final results whereas it was a poor prognostic sign in phakic detachment *in our opinion seems to support this thesis*

Summary and Conclusions

In a collection of 2 191 cases of retinal detachment two different series have *been studied* to explore the clinical features of aphakic retinal detachment

Although the number of aphakic *detachments is increasing the occurrence* of detachment in the aphakic eye is not increasing The real increase *was found* in the number of aphakic patients

Somewhat surprisingly the incidence of aphakic detachments originating from congenital cataracts was exactly the same in the two series to some extent a *contradiction* of the opinion that all aphakic eyes sooner or later will develop a detached *retina*

by far the most were excessive myopes Comparing the rate of reattachment we find it to be lowest in this latter group Whenever a cataract extraction in a juvenile patient with excessive myopia and with a reasonable amount of remaining vision is being considered the high risk of retinal detachment and the relatively poor success of reattachment should be borne in mind

In spite of a frequent overlapping of morphological characteristics in the phakic and aphakic retinal detachments there is sufficient clinical evidence for these to be considered as separate entities particularly in regard to extension location and shape of the detachment and the occurrence number and size of retinal breaks (Jaffe 1972)

It is a generally agreed that the aphakic detachment is usually of greater extent than primarily estimated with a preponderance of the lower quadrants and showing a flatter appearance than the phakic. These morphological features have not been studied in detail in this series As recorded in Table VIII only the occurrence of the retinal tear has been analyzed

It has been pointed out especially by Norton (1963) and Schepens (1950 1951) that in the aphakic detachment the breaks are multiple smaller and located closer to the ora than in the phakic detachment These characteristics which to some extent are confirmed by this study also lead directly to the last column of this Table the inability to find any tear

In this series no clear cut tear was found in 54 out of 187 cases i e 29%. This figure varies considerably in the reports published especially in the earlier papers The inability to find any tear ranged between 60% (Cordes

Table VIII

Type of cataract		Type of tear		
		U shaped	Multiple	No tear
Congenital	22	6	4	12 (51%)
Juvenile	38	15	18	9 (24%)
Senile	108	50	29	27 (25%)
Complicated	19	8	2	6 (32%)
Total	187	82	48	54

1956) and 40 % (Pasino & Santori 1962) Norton (1963) found no break in 16 % in contrast to Schepens (1951) who was unable to find any tear in only 3 % of the aphakic detachments Norton (1963) explains this discrepancy as being partly due to the small size of the breaks sometimes no larger than the caliber of a vessel and partly to the differences in interpretation of many suspect areas some investigators classified them as tiny breaks others as degenerative areas It is interesting to notice the differences among the four groups The figure of 51 % in the congenital group is depressing but explainable by the well known difficulty of visualising the fundus because of the small distorted pupil lens remnant opacities in the vitreous and frequently nystagmus In the complicated group vitreous haze plays a role but probably cases of exudative detachments also make their contribution It is however remarkable that no tear could be found in one fourth of the juvenile and senile aphakics even with the use of the current techniques of indirect binocular ophthalmoscopy and biomicroscopy with the Goldmann 3 mirror contact lens This might be due to lack of skill in the examination techniques and might further be explained by the different interpretations of a tear but in our opinion in the aphakic eye with the fundamental anatomical and physiological changes and frequent disturbances a particular type of retinal detachment with a different pathogenesis cannot be ruled out (Edmund 1964)

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Although the number of aphakic detachments is increasing the occurrence of detachment in the aphakic eye is not increasing The real increase was found in the number of aphakic patients

Somewhat surprisingly the incidence of aphakic detachments originating from congenital cataracts was exactly the same in the two series to some extent a contradiction of the opinion that all aphakic eyes sooner or later will develop a detached retina

Although certain clinical features separate aphakic detachment following congenital cataract from the others the reattachment rate was found to be astonishingly uniform

The importance of the surgical procedure in the cataract operation could not be appraised from this series The low reattachment rate following needling and extracapsular extraction is probably more a result of the difficulty in the examination and surgery of the detachment than a consequence of the cataract operation

The surgical procedure of choice in aphakic retinal detachment is encircling probably quite often combined with injection of hyaluronic acid into the vitreous

The occurrence of myopia before cataract extraction in aphakic detachments was found to be lower than among phakic detachments This seems to indicate the development of a pathological state of the peripheral choroid retina or vitreous along with the cataract and to emphasize a predisposition in the cataractous eye to be the fundamental primary factor in the occurrence of retinal detachment in the aphakic eye

The most dangerous type of aphakia as far as the occurrence of detachment is concerned appears following cataract extraction of a juvenile cataract in an excessively myopic eye The high risk of retinal detachment and the relatively poor success of reattachment should be borne in mind when cataract extraction of this type of patient is being considered

While the low incidence of tears found among the congenital and complicated aphakics is explainable the inability to discover a break in a quarter of the cases in the juvenile and senile groups is difficult to explain even taking into consideration the differences in the interpretation of a tear The possibility of a different pathogenesis in the origin of a retinal detachment in the aphakic eye is mentioned

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THE EFFECT OF THE CURVATURE OF THE EYE SHELL ON THE ECHOGRAM

BY

ARVO OKSALA and GEORG FRÄNKL

When sensitive ultrasonic equipment is used both clinical and experimental examinations reveal one or more low echoes in front of the high rear wall echoes if the sound beam is directed perpendicularly to the rear wall past the lens In the experimental part of the study 7 5 MHz/2 mm 6 MHz/5 mm and 6 MHz/8 mm transducers were used for the purpose of finding out the origin of these low echoes in pig eyes The results showed that these low echoes were due to the difference in distances between the central and marginal parts of the sound beam caused by the curvature of the eye shell Similar low echoes could be produced at clinical examinations at the frequencies of both 6 MHz and 10 MHz The low echoes disappeared when the amplification of our equipment was lowered from 80 db to [illegible] db Acoustic answers of low reflecting agents as slight dense and flat preretinal exsudates or haemorrhages therefore can not be distinguished from these findings described above

Key words ultrasound examination – sound beam – echogram – eye shell – rear eye wall – vitreous

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It has generally been assumed that the healthy vitreous of young and middle aged people is acoustically homogeneous Recent investigations with sensitive ultrasonic equipment have however revealed some low echoes in the vitreous spaces of these people (Lopping & Gartner 1968 Gartner & Lopping 1968 Nover & Kunde 1968) In addition observations with this type of equipment have shown that in some destructive conditions of the vitreous such as syneresis the vitreous space reflects several mobile echoes of varying height (Bellone 1968 Bellone & Gallenga 1971 Rossi & Gallenga 1971 Lopping Maratos & Nover 1971 Oksala 1973)

If we study the echograms presented by the above mentioned investigators we can often see one or more low echoes right in front of the rear wall echoes As we have examined the healthy vitreous spaces in different ages with the maximal sensitivity of our own ultrasonic equipment by directing the sound beam perpendicularly to the rear wall we have always been able to observe one or more low echoes in front of the high rear wall echo The origin of these echoes has been unknown to us In a discussion in 1968 Oksala suggested that the low echoes next to the rear wall echoes might be caused by the refraction of ultrasound at the lens in which case the margins of the sound beam would reach the eye wall earlier than the centre of the beam

In the present study we have investigated whether differences in distance between the central and marginal parts of the sound beam due to the curvature of the eye shell could produce the above mentioned low echoes also in cases where the examination is made past the lens or whether they arise from the denser cortex of the vitreous as anatomically described e g by G Eisner (1973)

Material and Methods

The examinations were made with Kretztechnik s A scan equipment model 100 and with four unfocused transducers of [illegible] 5 MHz/2 mm 6 MHz/5 mm [illegible] MHz 8 mm and 1[illegible] MHz/5 mm The first three transducers were used at experimental examinations and the last three for clinical purposes

Clinically we examined the healthy vitreous spaces of young middle aged and old people with the above mentioned transducers The low echoes immediately in front of the rear wall echoes were the sole object of our attention Among the examined persons 10 were under 20 years of age 10 between 40 and [illegible]0 and 10 between 60 and [illegible]0 The sound beam was directed past the lens perpendicular to the rear eye wall

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3A & B were obtained with the 6 MHz/5 mm transducer In Fig 3A the distance was 25 mm and one can see that there are low echoes in an approx 5 mm long area in front of the high scleral echoes When the distance from the transducer to the calotte was 15 mm (Fig 3B) these echoes are no longer observed The echograms in Fig 4 were obtained with the 6 MHz/8 mm transducer Fig 4A hows that at the distance of 25 mm low echoes extend 2 mm into the vitreous

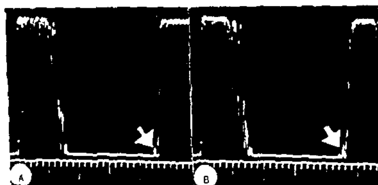
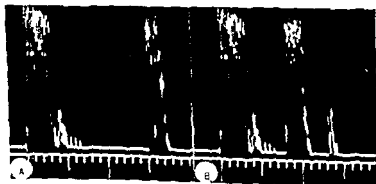


Fig 1

Figs A and B show 1 and 2 low e hoes (arrows) right in front of the high rear eye wall echoes at an exam nation made with the 6 MHz/5 mm transducer perpendicular to the rear wall The scale is showed in mm



Fig

Wh n the s und beam direct d with the 5 MHz 2 mm transducer perpendicular to a l r l rear wall cal tte of a *pig no low echoes can be observed* in front of the echoes reflected by the cal tt at the dist nce of 15 *mm (F g 2A)* nor at 8 mm (Fig 2B)

In the experimental part of the study we used eyes from newly slaughtered pigs Their equatorial diameter is approximately the same as that of human eyes i e about 23 mm They were prepared so that they consisted of a rear wall calotte of the sclera alone The wholly extracted vitreous was acoustically investigated *separately* *The calottes were placed on a metal* plate in a bowl filled with distilled water When the 7 5 MHz/2 mm transducer was used the calotte was however placed into a thin copper wire spiral so that the edges of the calotte were resting against the copper wire The transducers were fixed to a strand and the sound beam was directed as perpendicularly as possible to the bottom of the calotte The echograms reflected by the bottom of the calotte were produced with the 7 5 MHz/2 mm transducer when the distances between the transducer and the bottom were 8 and 15 mm and with the 6 MH /5 mm and 6 MHz/8 mm transducers at the distances of 15 and 25 mm

In order to clarify the possible effect of the edges of the calotte on the origin of the low echoes in front of the high rear wall echoes experiments were also made by cutting the edges off and by directing the sound beam to the bottom of the calotte which was only 5 mm in diameter These experiments were made with the 6 MHz/5 mm and 6 MHz/8 mm transducers

The diameters of the sound fields from the transducers used at the experimental investigations were measured at the maximal sensitivity of the equipment at the above mentioned distances A steel ball (diameter 1 5 mm) was used as a reflector which was moved via the acoustic centre of the sound field

Results

Clinical examinations revealed distinct differences in sensitivity between the *transducers Nevertheless the echograms of all the healthy* vitreous spaces examined contained one or more low echoes right in front of the high rear wall echoes Two examples of these can be seen in Fig 1 The 6 MHz/5 mm transducer was clearly the most sensitive The low echoes obtained by it extended 1–3 mm into the vitreous space while the other transducers produced echoes 1–2 mm in depth The low echoes always disappeared when the amplification of the equipment was lowered from the maximal 80 db to 72 db From the isolated pig vitreous these low echoes were not obtainable

Figs 2 3 & 4 represent echograms obtained at experimental examinations Fig 2 shows that at the distances of 8 and 15 mm the *7 5 MHz/2 mm transducer* reveals no echoes in front of the scleral echoes The echograms in Figs

3A & B were obtained with the 6 MHz/5 mm transducer In Fig 3A the distance was 25 mm and one can see that there are low echoes in an approx 5 mm long area in front of the high scleral echoes When the distance from the transducer to the calotte was 15 mm (Fig 3B) these echoes are no longer observed The echograms in Fig 4 were obtained with the 6 MHz/8 mm transducer Fig 4A shows that at the distance of 25 mm low echoes extend 2 mm into the vitreous

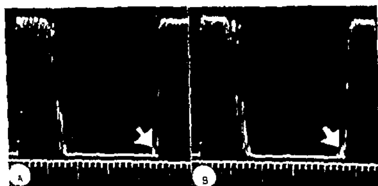


Fig 1

Figs A and B show 1 and 2 low echoes (arrows) right in front of the high rear eye wall echoes at an examination made with the 6 MHz 5 mm transducer perpendicular to the rear wall The scale is showed in mm

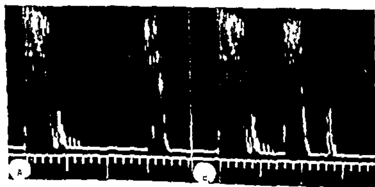


Fig 2

Wh n th und bea n is directed with the .5 MHz 2 mm transducer perper dicular to a leral r ar wall al tte f a pig no low echoes can be observed in front of the echoes fl ted by th l tte at the distance of 15 mm (F g 2A) nor at 8 mm (Fig 2B)

In the experimental part of the study we used eyes from newly slaughtered pigs. Their equatorial diameter is approximately the same as that of human eyes, i.e. about 23 mm. They were prepared so that they consisted of a rear wall calotte of the sclera alone. The wholly extracted vitreous was acoustically investigated separately. The calottes were placed on a metal plate in a bowl filled with distilled water. When the 7.5 MHz/2 mm transducer was used the calotte was, however, placed into a thin copper wire spiral so that the edges of the calotte were resting against the copper wire. The transducers were fixed to a strand and the sound beam was directed as perpendicularly as possible to the bottom of the calotte. The echograms reflected by the bottom of the calotte were produced with the 7.5 MHz/2 mm transducer when the distances between the transducer and the bottom were 8 and 15 mm, and with the 6 MHz/5 mm and 6 MHz/8 mm transducers at the distances of 15 and 25 mm.

In order to clarify the possible effect of the edges of the calotte on the origin of the low echoes in front of the high rear wall echoes, experiments were also made by cutting the edges off and by directing the sound beam to the bottom of the calotte which was only 5 mm in diameter. These experiments were made with the 6 MHz/5 mm and 6 MHz/8 mm transducers.

The diameters of the sound fields from the transducers used at the experimental investigations were measured at the maximal sensitivity of the equipment at the above mentioned distances. A steel ball (diameter 1.5 mm) was used as a reflector which was moved via the acoustic centre of the sound field.

Results

Clinical examinations revealed distinct differences in sensitivity between the transducers. Nevertheless the echograms of all the healthy vitreous spaces examined contained one or more low echoes right in front of the high rear wall echoes. Two examples of these can be seen in Fig. 1. The 6 MHz/5 mm transducer was clearly the most sensitive. The low echoes obtained by it extended 1–3 mm into the vitreous space, while the other transducers produced echoes 1–2 mm in depth. The low echoes always disappeared when the amplification of the equipment was lowered from the maximal 80 db to 72 db. From the isolated pig vitreous these low echoes were not obtainable.

Figs. 2, 3 & 4 represent echograms obtained at experimental examinations. Fig. 2 shows that at the distances of 8 and 15 mm the 7.5 MHz/2 mm transducer reveals no echoes in front of the scleral echoes. The echograms in Figs.

Table 1
The diameters of the sound fields of the various transducers used at the experimental examinations measured at different distances

Transducer	Distance between the transducer and the steel ball	Diameter of the sound field
7.5 MHz/2 mm	8 mm	9 mm
7.5 MHz/2 mm	15 mm	11 mm
6 MHz/5 mm	15 mm	15 mm
6 MHz/5 mm	25 mm	17 mm
6 MHz/8 mm	15 mm	17 mm
6 MHz/8 mm	25 mm	18 mm

The diameters of the sound fields of the various transducers used at the experimental examinations were afterwards measured at the above mentioned distances. Table 1 shows the obtained results.

Discussion

Clinical examinations showed that with the maximal intensity of the equipment and with sensitive transducers one or more low echoes could always be obtained in front of the high rear wall echoes at a perpendicular examination. These low echoes extended 1–3 mm into the vitreous space. They could be produced at the frequencies of both 6 MHz and 10 MHz, which shows that their appearance was independent of the frequency. Their number, however, was clearly dependent on the sensitivity of the equipment and the transducer.

At an experimental examination these low echoes in front of the high rear wall echoes could not be obtained by the 7.5 MHz/2 mm transducer, but with the 6 MHz/5 mm and 6 MHz/8 mm transducers they were always obtainable provided the distance between the transducer and the calotte was sufficient, i.e. 25 mm. If the distance was only 15 mm, these echoes disappeared. Neither could they be produced if the edges of the calotte were removed so that the sound beam met only an almost level bottom of the calotte with a diameter of

in front of the high echoes reflected by the calotte When the distance is reduced to 15 mm in Fig 4B these low echoes are no longer visible. After the edges of the calotte had been cut off and only part of the calotte bottom 5 mm in diameter was left no low echoes could be produced in front of the echoes from the calotte bottom with the 6 MHz/5 mm and 6 MHz/8 mm transducers at 25 mm distance

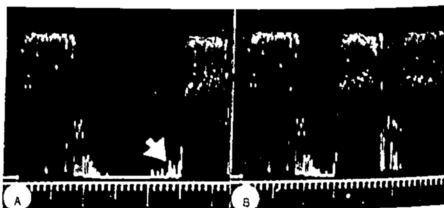


Fig 3
At the distance of 25 mm the 6 MHz 5 mm transducer produces several low echoes in front of the high echoes reflected by the calotte These low echoes extend 5 mm into the vitreous (Fig 3A arrow) but at the distance of 15 mm no low echoes are visible (Fig 3B)

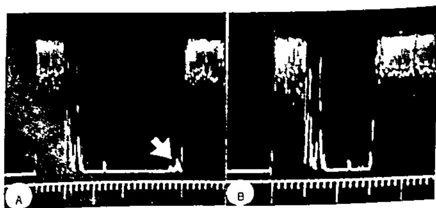


Fig 4
At the distance of 25 mm with the 6 MHz 8 mm transducer the low echoes extend 7 mm in front of the high echoes reflected by the calotte (Fig 4A arrow) while no low echoes can be seen at the distance of 15 mm (Fig 4B)

Table 1

The diameters of the sound fields of the various transducers used at the experimental examinations measured at different distances

Transducer	Distance between the transducer and the steel ball	Diameter of the sound field
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7.5 MHz/2 mm	15 mm	11 mm
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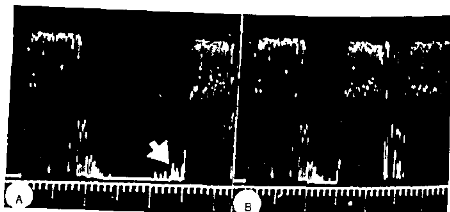


Fig 3
At the distance of 25 mm the 6 MHz/5 mm transducer produces several low echoes in front of the high echoes reflected by the calotte These low echoes extend 5 mm into the vitreous (Fig 3A arrow) but at the distance of 15 mm no low echoes are visible (Fig 3B)

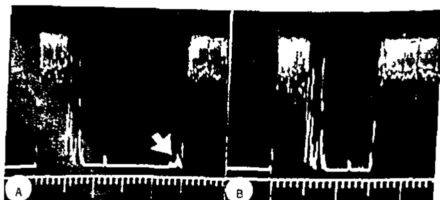


Fig 4
At the distance of 25 mm with the 6 MHz 8 mm transducer the low echoes extend 2 mm in front of the high echoes reflected by the calotte (Fig 4A arrow) while no low echoes can be seen at the distance of 15 mm (Fig 4B)

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5 mm The fact that low echoes were received at experimental examinations even 5 mm from the bottom of the calotte can be explained by the fact that the curvature of the edges of the calotte differs from the curvature of the whole eye

It is our opinion that the clinical and experimental examinations show quite clearly how the curvature of the eye shell is at least one factor which produces one or more low echoes in front of the high rear wall echoes If the transducer is sensitive enough and if the sound field is sufficiently large in diameter the low echoes reflected in the margins of the sound beam will appear on the screen earlier than the high echoes reflected in the centre The distances of the smallest echoes from the high rear wall echoes i e 1–3 mm correspond well to the difference in distances between the central and marginal parts of the sound beam from the transducer to the sclera

The clinical significance of the above results could be expressed as follows If sensitive equipment is used for examining pathological processes near the rear wall the screen may contain some low echoes which are due to the difference in distances between the central and marginal parts of the sound beam caused by the curvature of the rear wall These low echoes will however disappear from the screen when the intensity of the equipment is lowered In our own equipment the required lowering was of the order of 5–8 db In this way it does not seem to be possible to decide 1) whether the region of the cortex of the vitreous is acoustically homogeneous or not 2) small and slightly dense preretinal haemorrhages or exsudates can not be recognized with certainty

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Since Karpe (1945) introduced a clinical method for recording the electroretinogram (ERG) from the human eye the method has been widely used It allows the assessment of the general function of the retina in cases in which the media are opaque For this reason it is of special value in conditions such as congenital cataract because the degenerative changes may in some cases involve the retina It has been found that there is a relationship between the amplitude of the b potential in the preoperative electroretinogram recorded according to Karpe s methods and the operative results It may therefore reasonably be assumed that in cases of congenital cataract in which surgery is under consideration the preoperative ERG should afford valuable diagnostic information about the therapeutic results of the operation

The main object of the present investigation was to ascertain whether there was a relationship between the amplitude of the b pot in the preoperative ERG and the therapeutic results in cases of congenital cataract

The original series comprised 162 patients with congenital cataract who were examined by ERG prior to surgery Seventy eight patients were excluded for various reasons One reason was that the visual acuity was unmeasurable after the operation Another reason was that communication with the patient was difficult or impossible on account of the tender age mental retardation or deafness of the patient The remaining 84 cases (114 eyes) form the basis of this investigation

Several earlier investigators studied the ERG in congenital cataract François (19 3) reported five cases of congenital cataract in which the patients were 2 to 3 months old The ERGs of four of these patients were normal corresponding t their ages that of the remaining patient was unrecordable The ERGs of a further five patients whose ages ranged from 5 to 9 months were likewise normal corresponding to their ages In a group of 30 cases of congenital cataract in which the patients were over 1 year or adults he found the ERGs to be normal in 16 subnormal in 10 and unrecordable in four patients Shappert Kimmijser (1958) examined 13 patients with congenital cataract by electroretinography prior to surgery The ERGs were subnormal in eight and normal in five of these cases Although the operation was successful from a technical point of view the patients postoperative visual acuity was poor Henkes & Shappert Kimmijser (19 0) examined by ERG 84 partially sighted children who had previously been operated for congenital cataract. In eight of these cases the cataract was due to an infectious disease in 28 the condition was hereditary and in 48 cases the aetiology was unknown Henkes & Shappert Kimmijser divided these cases according to the type of ERG recorded into cases with a normal subnormal or unrecordable ERG and found the postoperative visual acuity to be as follows

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THE ELECTRORETINOGRAM IN CONGENITAL CATARACT

BY

B. ZETTERSTRÖM

A total of 114 eyes affected with congenital cataract were examined by electroretinography prior to surgery. The preoperative electroretinogram (ERG) of 91 eyes was normal, of 17 eyes subnormal and in six eyes it was unrecordable.
There was a clear cut relationship between a subnormal preoperative ERG and a poor postoperative visual acuity: of the 17 eyes of which the preoperative ERG was subnormal, the postoperative visual acuity of 12 eyes was < 0.1 and of the remaining eyes 0.1–0.2. In the cases in which the preoperative ERG is unrecordable, the chance of restoring a good postoperative visual acuity by surgery is very small. The visual acuity of the six eyes in this series from which the preoperative ERG was unrecordable was < 0.1.
The therapeutic possibilities of surgery cannot invariably be conclusively assessed from the normal preoperative ERG because the latter does not reflect possibly coexisting macular lesions or lesions proximal to the retina. Nevertheless, in cases in which the preoperative ERG is normal, the chance of achieving good therapeutic results with surgery is greater: out of 91 eyes in this series from which a normal preoperative ERG was recorded, the postoperative visual acuity of 10 eyes was < 0.1, of 36 eyes 0.1–0.2 and of 45 eyes 0.3 or more.

Key words: cataracta congenita – electroretinography (ERG).

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the b wave as shown in the ERGs of 74 healthy eyes and arrived at the conclusion that *a b wave exceeding* 0.54 0.18 mV is almost certainly abnormal. Peterson (1968) studied the b pot in the ERGs of 322 healthy eyes and reported the following values

The b potential in 178 men aged 10–69 years

Age years	mV Mean	Limits including certain percentage of normal cases (mV)		
		95 %	99 %	99.9 %
10–19	0.39	0.23–0.55	0.18–0.60	0.12–0.66
20–29	0.37	0.23–0.53	0.16–0.58	0.10–0.64
30–39	0.36	0.20–0.52	0.15–0.57	0.09–0.63
40–49	0.34	0.18–0.50	0.13–0.55	0.0 –0.61
50–59	*0.33*	*0.17–0.49*	0.12–0.54	0.06–0.60
60–69	0.32	0.16–0.48	0.11–0. 3	0.05–0.59

The b potential in 144 women aged 10–69 years

Age years	mV Mean	Limits including certain percentage of normal cases (mV)		
		9 %	99 %	99.9 %
10–19	0.42	0. 6–0. 8	0.21–0.63	0.15–0.69
20–29	0.39	0.23–0.55	0.18–0.60	0.12–0.66
30–39	0.41	0.25–0. 7	0.20–0.62	0.14–0.68
40–49	0.44	0. 5–0.60	0.23–0.65	0.1 –0.71
50–59	0.35	0.19–0.51	0.14–0.56	0.08–0.62
60–69	0.35	0.19–0.51	0.14–0.56	0.08–0.62

(C p d fr m I ter n 1968)

It is seen that the values of the b pot reported by Peterson are in agreement with those reported by Karpe. As the conditions under which the patients in this series were examined were the same as those under which Peterson and Karpe respectively examined their patients, the lower limit of the normal amplitude of the b pot was set at > 0.20 mV. An ERG showing this amplitude of the b wave was considered as normal

Correlation of the alterations in visual acuity with those found in the ERG

Etiology of connatal cataract	Normal ERG No of eyes showing a visual acuity of		Subnormal or absent ERG No of eyes showing a visual acuity of	
	< 1/10	1/10 to 3/10	< 1/10	1/10 to 3 10
Infectious (12 eyes)	7	2	3	0
Hereditary (32 eyes)	16	11	4	1
Unknown (53 eyes)	20	23	10	0

(Copied from Henkes and Shappert Kimmijser 1960)

It is seen that the visual acuity of the eyes whose ERGs were subnormal or unrecordable was very poor being > 0 1 in only one eye out of 18 eyes in the other 17 eyes it was < 0 1 Out of 79 eyes whose ERGs were normal the visual *acuity of 43 eyes was < 0 1 and that of 36 eyes ranged from 0 1 to 0 3* The probable explanation of the poor visual acuity of these patients is that they were selected from a group of partially sighted children

Material and Method

A total of 114 eyes affected with different types of congenital cataract were examined by ERG and subsequently operated upon The operative methods were discission which was performed on several occasions direct aspiration of the eye lens or intracapsular extraction which was carried out in only a few cases The postoperative visual acuity was measureable in *all* these cases *The* ERG was recorded according to Karpe s method (1948) The stimulus light was 20 80 and 800 Lux respectively the latter stimulus light being used to enable a b pot of maximum amplitude to be recorded in all cases even in the presence of a strongly light absorbing lens (Karpe & Vainio Mattila 1951) The ERG was assessed on *the basis of the maximum amplitude of the b pot irrespective* of the intensity of the stimulus light used Children below the age of 10 were usually examined under anaesthesia An ERG showing the amplitude of the b wave to be 0 20 mV or higher was considered as normal Karpe (1945) studied

The postoperative visual acuity was < 0 1 in all cases Although there were no complications from the operation and seven of the patients were given more or less intensive treatment of amblyopia the postoperative visual acuity was very poor in all these cases

Comments

In cases of congenital cataract in which a subnormal ERG is recorded especially in those in which no electrical response is elicited the chance of restoring good visual acuity by surgery is very small While the retinal layer in which the components of the ERG originate is still not quite clear there is no doubt that the degenerative changes involve a large portion of the retinal surface and not only the macular region in cases of congenital cataract in which the ERG is subnormal or unrecordable It is well known that the ERG recorded by the method used in this investigation does not reflect localized lesions The ERG reflects a summation response from the illuminated retinal surface

On the other hand a normal ERG does not invariably afford reliable prognostic information about the presumed therapeutic possibilities of the operation As mentioned above the ERG recorded by the method used in this investigation does not reflect localized lesions of the macula This applies also to lesions proximal to the retina

Table I shows that of 91 eyes whose preoperative FRG was normal the postoperative visual acuity of only 10 was < 0 1 This suggests that eyes whose electrical response is normal carry on average a considerably better prognosis in relation to the postoperative visual acuity However it should be kept in mind that the postoperative visual acuity may eventually decline in the long run even in cases in which the preoperative ERG was normal owing to possible late complications such as retinal detachment

As the amplitude of the b pot increases in infants during the first years of life it is of major importance to perform electroretinography on several occasions during that period otherwise it cannot be determined with assurance whether the ERG is normal or subnormal (Zetterstrom 1951 1956 Algvere & Zetterstrom 1967)

The prognostic value of the preoperative ERG in cases of congenital cataract in which surgery is under consideration is obvious it may be helpful in assessing the therapeutic possibilities of the operation

In unilateral congenital cataract the preoperative ERG appears to be of lesser prognostic value in relation to the postoperative visual acuity because the affect

As already mentioned, the main purpose of this investigation was to ascertain whether there was a relationship between the amplitude of the b-wave as shown in the preoperative ERG and the postoperative visual acuity. For this reason the cases were not divided according to the type of cataract present.

It is a well-known fact that congenital zonular cataract carries a far better prognosis in relation to the postoperative visual acuity than total congenital cataract. The investigation was extended to include 16 children with unilateral congenital cataract, their ages ranging from 8 months to 4 years. They were all examined by ERG prior to surgery and their postoperative visual acuity was measured.

Results

Table I shows the type of preoperative ERG and the postoperative visual acuity of the 114 eyes examined.

In the group of 91 eyes whose ERGs were normal, the ages of 14 patients ranged from 19–47 years and the ages of 77 patients from 7 months to 9 years. In the group of 17 eyes from which a subnormal ERG was recorded, the patients were aged 7 months to 6 years and in the group of six eyes from which no electrical response was elicited the patients' ages ranged from 7 months to 2 years. In the group of 16 children with unilateral congenital cataract the ERG of the healthy eye was normal, that of the affected fellow eye being normal in 12 and subnormal in two patients; in the remaining two patients the ERGs were unrecordable.

Table I

Type of ERG	Postoperative visual acuity		
	< 0.1	0.1–0.2	0.3 and more
Normal (91 eyes) (> 0.20 mV) 79.8 %	10	36	45
Subnormal (17 eyes) (< 0.20 mV) 15.0 %	12	5	0
Unrecordable (6 eyes) 5.2 %	6	0	0

The postoperative visual acuity was < 0 1 in all cases Although there were no complications from the operation and seven of the patients were given more or less intensive treatment of amblyopia the postoperative visual acuity was very poor in all these cases

Comments

In cases of congenital cataract in which a subnormal ERG is recorded especially in those in which no electrical response is elicited the chance of restoring good visual acuity by surgery is very small While the retinal layer in which the components of the ERG originate is still not quite clear there is no doubt that the degenerative changes involve a large portion of the retinal surface and not only the macular region in cases of congenital cataract in which the ERG is subnormal or unrecordable It is well known that the ERG recorded by the method used in this investigation does not reflect localized lesions The ERG reflects a summation response from the illuminated retinal surface

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REPORT OF A CASE OF RETINITIS PIGMENTOSA FOLLOW UP FROM 7 MONTHS TO 27 YEARS OF AGE

BY

BIRGITTA ZETTERSTRÖM

A case of dominant retinitis pigmentosa (r p) is described in which the presumptive diagnosis of the condition was made when the patient was months old solely from the finding that the ERGs from both eyes were unrecordable other characteristic ophthalmoscopic changes being absent When the patient was 27 years old the ERGs of both eyes were still unrecordable and this together with the now established typical changes of the fundi the abnormal shape of the dark adaptation curve and the characteristic field defects confirmed the earlier presumptive diagnosis of r p

Key words retinitis pigmentosa electroretinography (ERG)

In 1951 Björk and Karpe reported a series of cases of tapeto retinal degeneration in which the patients were examined by electroretinography The series included a month old boy who had a family history of dominant retinitis *pigmentosa (r p) Ophthalmoscopically* there was no conclusive evidence of the condition The findings are quoted from Björk & Karpe (1951) C L a male *patient* was born on Oct 21th 1946 his mother had dominant r p Ophthalmoscopic examination of the patient one day after birth did not reveal any changes characteristic of r p However ophthalmoscopic examination months later showed the fundus to be pale *with traces of small white spots* otherwise no abnormality was found The electroretinogram (ERG) *using* 20 mc as light

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ed eye tends to develop amblyopia even in cases in which a normal preoperative ERG was elicited

In the 16 cases of unilateral congenital cataract in which the patients were operated upon the postoperative visual acuity was < 0 1

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Fig 1
Pigment deposit of the left fundus

stimulus was unrecordable When the patient was 3 years old there was ophthalmoscopic evidence of numerous light spots in the periphery of the fundus some showed pigmented borders others had a pigmented dot in their centre The discs maculae and blood vessels were normal Thus the fundus showed pathological changes which at that stage were not typical of r p Orientation tests in

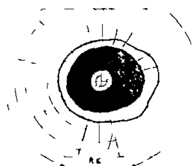


Fig 2
Visual field of the right eye

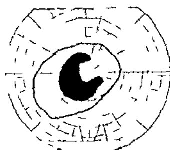


Fig 3
Visual field of the left eye

semi darkness suggested reduced light sense The ERG s using light stimuli of 20 and 80 m.c respectively were extinguished

In view of the patient s family history of dominant r p the presence of r p was already strongly suggested at that time The bilateral absence of the ERG was considered to be sufficiently pathognomonic to enable the presumptive diagnosis to be made with a reasonable degree of assurance even at the tender age of the patient despite the absence of fundus abnormalities field defects

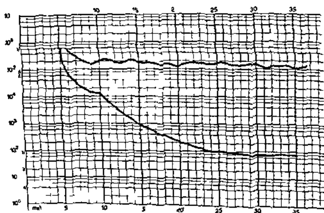


Fig 4
Upper curve shows the dark adaptation of the patient with Goldmann Weekers adaptometer Lower curve shows upper border line of the normal range in healthy individuals of the same age as the patient



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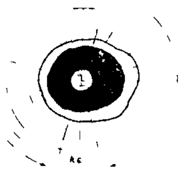


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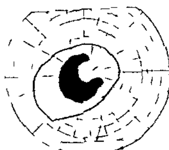


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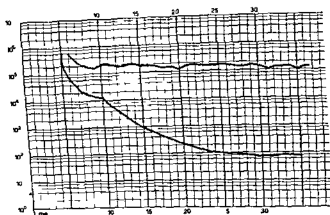


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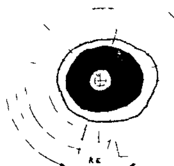


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Visual field of the right eye

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LUMINOSITY FUNCTIONS OF THE OSCILLATORY POTENTIALS OF THE HUMAN ELECTRORETINOGRAM

BY

LILLEMOR WACHTMEISTER

M nochromatic light at 13 selected points of the spectrum was presented to the eyes of three normal subjects Spectral sensitivity curves for the slow (a and b waves) as well as the rapid (oscillatory) potentials were determ ned in response to light stimuli delivered in conditions of com paratively (1) strong light adaptation induced by light stimuli (at an inter val of 15 sec) and (2) by weak light adaptation induced by light stimuli (at an interval of 2 min)

From comparisons of the sensitivity functions obtained it was concluded that while there was a change of retinal sensitivity from predominantly photopic to scotopic type no variation of the spectral sensitivity of the o cillations was recorded

In photopic as well as scotop c conditions studied the oscillations *revealed two peaks of sensitivity at 472 nm and 551 nm of the spectrum* Thus the results seem to support earlier suggestions of the oscillatory potentials being related to an interaction by rod and cone activities and the or gin of the oscillations being quite separate from that of the a and b wave

Key words electroretinography – oscillatory potentials – luminosity functions – scotop c and photopic conditions

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typical of r p and reduced dark adaptation

The patient who at present is 27 years old was again examined in 1973 with *special reference to the presence of r p* He is working as an engineer and apart from night blindness has no visual incapacity The visual acuity of his right eye was 0 7 (+4 5–0 5 c 100°) *that of the left one being* 0 9 (+4 5–0 5 c 180) He can read Jaeger s test type No 1 with his refraction for distance

Both fundi showed very narrow vessels waxy atrophy of the optic nerve being suggested Numerous deposits of pigment were present in the periphery of the fundi their appearance was typical of r p resembling bone corpuscle a slightly larger number being present in the left fundus (Fig 1)

Visual fields according to Goldmann, revealed slight concentric contraction and big ring scotomata and big cylindrical scotomata in their centres (Figs 2 3)

The dark adaptation curve as measured with the Goldmann Weekers adaptometer was abnormal being higher by about 3 log units than normal (Fig 4) As in 1947 and 1949 the ERGs of both eyes elicited according to Karpe (1945) using light stimuli of 20 80 and 800 Lux respectively were extinguished *In* addition electron flashes were used as light stimuli which likewise failed to elicit an electrical response

Comments

The results of the present examinations conclusively confirmed the presence of r p However the diagnosis could already have been made with a reasonable degree of confidence when the absence of the electrical response of both eyes was noticed when the patient was 7 months old despite the absence of typical ophthalmoscopic appearances

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LUMINOSITY FUNCTIONS OF THE OSCILLATORY POTENTIALS OF THE HUMAN ELECTRORETINOGRAM

BY

LILLEMOR WACHTMEISTER

Monochromatic light at 13 selected points of the spectrum was presented to the eyes of three normal subjects Spectral sensitivity curves for the slow (a and b waves) as well as the rapid (oscillatory) potentials were determined in response to light stimuli delivered in conditions of comparatively (1) strong light adaptation induced by light stimuli (at an interval of 15 sec) and (2) by weak light adaptation induced by light stimuli (at an interval of 2 min)

From comparisons of the sensitivity functions obtained it was concluded that while there was a change of retinal sensitivity from predominantly photopic to scotopic type no variation of the spectral sensitivity of the oscillations was recorded

In photopic as well as scotopic conditions studied the oscillations revealed two peaks of sensitivity at 472 nm and 551 nm of the spectrum Thus the results seem to support earlier suggestions of the oscillatory potentials being related to an interaction by rod and cone activities and the origin of the oscillations being quite separate from that of the a and b wave

Key words electroretinography – oscillatory potentials – luminosity functions – scotopic and photopic conditions

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Under dark adapted conditions the spectral sensitivity curve for the human LRG (a and b wave) shows a peak near 500 nm and a close agreement with psychophysical determination of luminosity (the CIL scotopic luminosity function) (Riggs Berry & Wayner 1949 Armington Johnson & Riggs 1952 Granada & Biersdorf 1966 van Lith 1966) In light adaptation the peak sensitivity of the eye shifts to longer wave length which has been shown in electrophysiological and psychophysical studies (Armington 1955 Armington & Biersdorf 1956 Biersdorf & Armington 1952 van Lith 1966 Samson Dollfus 1969) The shape of the ERG spectral curve conforms closely to the CIL photopic luminosity function (van Lith 1966 Johnson, Riggs & Schick 1966 Adams & Dawson 1971)

In 1967 Heck & Rendahl suggested that the oscillatory potentials may reflect activity of specific spectral mechanisms Nye (1968) on the other hand could not support the notion that the oscillations of the pigeon s ERG are related to the activity of independent colour mechanisms of retina Rouher Sole & Alfieri (1966) recorded the most prominent oscillatory potentials in response to long wave length stimulus whereas on the short wave length record only the slow potentials of the human (a- and b wave) were noticeable However Ardouin Garnier & Herve (1970) reported oscillations of the human eye were most easily elicited in response to blue and green stimuli whereas they were hardly recordable in response to red light Adams & Dawson (1971) studied a fast retinal potential (FRP) of the human eye related to the oscillatory potentials in response to stimuli of equal energy In mesopic conditions the FRP was much larger when elicited by stimuli of 500 nm than recorded in response to 560 nm (or longer) wavelengths During photopic conditions the FRP showed a photopic spectral sensitivity function Studies of the oscillatory potentials of the human ERG during light and dark adaptation showed the oscillations to be most easily elicited during mesopic condition at the level of retinal sensitivity where the shift from photopic to scotopic vision or vice versa occurs (Algvere & Wachtmeister 1972 Wachtmeister 1973 a b)

As no systemic study has been published regarding thresholds relation to monochromatic stimulus intensity and the spectral sensitivity of the oscillatory potentials the primary purpose of the present investigation was to determine the relative luminous efficiency function of the oscillatory potentials of the human ERG

Apparatus and Methods

The photostimulator was an Oriel xenon dc arc lamp (XBO 900 w/2 Osram)

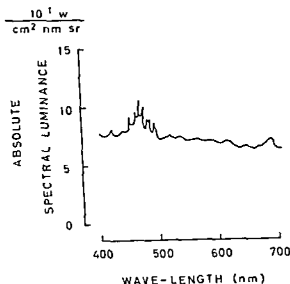


Fig 1
Spectral d stribution of the light from the Oriel xenon arc lamp (900 W)

powered by a regulated power supply (Jovy) which supplied a constant current to the arc The light from the arc passed through a water chamber a series of optical lenses and apertures which passed only light from the hottest part of the arc The maximum luminance of the white light beam used was about 1 0 x 10^6 photopic cd/m^2 measured with a Hagner Universal Photometer Its colour temperature corresponded to about 6 000 K and its spectral distribution was similar to the solar spectrum (Fig 1) The duration of light stimulus was con trolled by an electromagnetic shutter Its rise time is about 1 msec The duration of stimulus was set to 25 msec

The light was then presented to the eye reflected by a mirror as a slightly divergent beam of about 10 cm in diameter after having passed through ap propriate narrow (16-24 nm) band interference filters (Schott λ maximum varying between 404 to 705 nm) and neutral density wedges

Energy levels of each of the narrow band chromatic stimuli were determined and calibrated using a radiation thermoelement The λ maxima of the interfe rence filters used are 404 425 450 472 499 522 551 579 596 628 646 676 and 705 nm

The electric response was recorded and amplified using the same electronic equipment calibration and bandpass of recording system as previously described (Algvere Wachtmeister & Westbeck 1972) A Lawwill Burian contact lens (Lawwill & Burian 1966) was used as an active and reference electrode respectively The ground electrode was attached to the ear The electric signal was displayed on a double beam cathode ray oscilloscope (Hewlett & Packard 132 A) and photographed One beam presented the slow ERG response (a and b wave) in a low speed simultaneously with the rapid response (oscillatory potentials) in a high speed time scale on the other

The methods of measurement of the amplitudes were previously described by Algvere et al 1972

Procedures

Three young and healthy adults served as subjects The visual acuity visual fields adaptometric visual sensitivity and colour sense were normal The ERG pictures shown were all recorded from the right eye of the same subject

Topical anesthesia of the eye was induced by Novesin® (Wander) and the pupil dilated to more than 6 mm in diameter The fellow eye was occluded

The ERG was recorded after at least 30 min of dark adaptation The following two procedures were performed

1 Light stimulus was given in a series of three with intervals of 15 sec The first two stimuli in each series induced a comparatively strong state of light adaptation at the point of time when the third stimulus light was delivered The ERG was recorded in response to this third stimulus light The intensity of all three stimuli was increased in steps of 0 3–0 4 log units varying over 5 log units There was an interval of 15 sec between each series of three light stimuli The procedure was repeated with monochromatic stimuli of 13 different wavelengths varying between 404–705 nm

2 In another series of experiments very similar to the procedure described above light stimuli were given with longer intervals i e 2 min The light adaptation caused by previous flashes was then comparatively weak when the next stimulus appeared The ERG was recorded as the stimulus intensity was increased logarithmically at steps of 0 3–0 4 logarithms

Results

1 Strong light adaptation caused by stimulus light

The thre hold and *spectral sensitivity* function of the a and b wave and the oscillatory potentials of the ERG were studied The threshold of the FRG was recorded in response to light stimulus of 13 different wave lengths varying between 404 to 705 nm Three consecutive light stimuli of same intensity were given and the ERG recorded *in response to the third* stimulus light. The intensity of all three lights varied from log I_s=-5 0 to log I -0 The light stimuli were delivered every 15 sec which caused a comparatively strong light adaptation of the retina

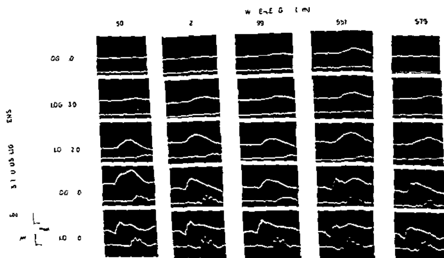


Fig 2

Threshold and relation to stimulus light intens ty of the amplitude of the a and b wave and the oscillatory potentials on stimulation of lights of different wave lengths The wave length was varied between 404 705 nm A series of three st mulation lights was given The oscillatory potentials and the slow potentials were recorded simultaneously in response to the third (st mulus) l ght Each ERG was displayed in a slow cathode ray speed (upper trace) and simultaneously in a rapid speed (lower trace) The intensity of all three lights increased n a logarithmic scale. There were 15 sec between each light although the intensity *of the lights varied Five selected wave lengths* of an equal energy spectrum at 5 different intens ties are illustrated (450 47? 499 551 and 579 nm)

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2 In another series of experiments very similar to the procedure described above light stimuli were given with longer intervals i e 2 min The light adaptation caused by previous flashes was then comparatively weak when the next stimulus appeared The ERG was recorded as the stimulus intensity was increased logarithmically at steps of 0 3–0 4 logarithms

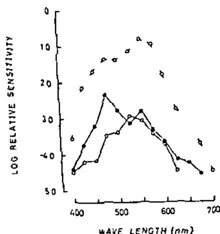


Fig 3

Spectral sensitivity (reciprocity of threshold intensity) function of the a and b wave and the oscillatory potentials in response to lights of 13 different wave lengths The procedure was the same as in Fig 1 Threshold criterion was 33 μV for the a and b wave and .5 a u (arbitrary units) for the oscillatory potentials The log relative sensitivity (reciprocity of threshold) plotted against wave length of light stimulus on the x axis Average of three subjects Black circles oscillatory potentials Open circles continuous line a wave Open circles dashed line b wave.

closely departing most widely at 400 nm and at 628 nm and longer wave lengths (Fig 3)

The oscillatory potentials showed a relative increase in sensitivity at 472 and 551 nm respectively The shape of the derived sensitivity function disagreed with the standardized functions of the photopic as well as scotopic luminosity function (Commission International de L Eclairage (1957)

II Weak light adaptation caused by stimulus light

The ERG was recorded in response to stimulus light elicited at an interval of 2 min which induced a comparatively weak light adaptation to retina The threshold and spectral sensitivity function of the a and b wave and the oscillatory potentials of the ERG were studied using 13 different wave lengths of stimulus light The results were evaluated and compared to those obtained when stimulation intervals of 15 sec were used

ERG thresholds

A b wave of about 120 μV appeared at stimulus light intensity of log I_s=-4 6 on stimulation with light of wave length 551 nm Using stimuli of 450 472 499 and 579 nm the b wave was recorded at stimulus intensity of more than log I_s=-4 0 (Fig 2) An a wave of 33 μV was recordable when stimulus intensity was log I_s=-2 3 and light of 551 nm was used Stimulation with monochromatic light of 450 472 499 and 579 nm did not evoke a distinct a wave until the stimulus intensity was log I_s=-1 6 or stronger (Fig 2)

The oscillatory potentials were recorded at stimulus of log I_s=-2 6 using a wave length of 472 nm Stimulating with lights of 450 551 and 579 nm did not elicit any distinct oscillations until the stimulus intensity was log I =-2 0 or stronger A stimulus intensity of log I_s=-1 6 or stronger was necessary to evoke the oscillations on the 499 nm record (Fig 2)

Consequently the threshold of the a and b wave was lowest using light of 551 nm wave length whereas the oscillatory potentials were most easily elicited when stimulating with wave length of 472 nm Most prominent oscillatory potentials were recorded in response to maximum stimulus intensity on the 450 472 and 499 nm record

Spectral sensitivity function

In the present electroretinographical study the relative* sensitivity (reciprocity of threshold) of the eye at 13 different wave lengths of the spectrum was measured This spectral sensitivity expresses the differences in intensity of light stimulus necessary to elicit a constant (criterion) electrical response when lights of different wave length are used The criterion voltage of the slow potentials (a and b wave) was 33 μV A criterion amplitude of 2 5 arbitrary units (a u) was used for the oscillatory potentials Threshold curves of the neutral filtering required for criterion response at each tested wave length were plotted Spectral sensitivity curves were derived for both a and b waves and the oscillatory potentials

The spectral sensitivity function of the b wave gave a fairly good approximation of the human photopic spectral sensitivity function for peripheral cones with maximum sensitivity at about 551 nm (Wald 1945) The major departures were at 628 nm and above (Fig 3)

The maximum sensitivity of the a wave is found to be at 522 551 nm and the shape of function approximates the photopic luminous efficiency curve rather

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*) relative in the sense that no constitution of a ratio scale with reference to an absolute energy calibration was made

Spectral sensitivity function

The sensitivity (reciprocity of threshold) of the eye was determined measuring the electroretinal threshold at various wave lengths Spectral sensitivity curves were derived for the a and b wave and the oscillatory potentials in the same manner as described on page 358

The spectral sensitivity functions displayed in Fig 5 discloses the function for the a and b wave to give a fairly good approximation to the human scotopic spectral sensitivity curve for peripheral rods and show a peak at about 499 nm (Wald 1945) Most disagreement to the CIE scotopic luminosity function occurred at 678 nm and above where the function of the a and b wave is somewhat elevated

The maximum sensitivity of the oscillatory potentials occurred at 472 and 551 nm It declines at both sides and in between revealing a double sensitivity

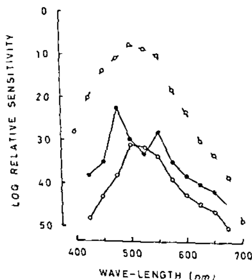


Fig 5

Spectral sensitivity (reciprocity of threshold intensity) function of the a and b wave and the oscillatory potentials in response to lights of 13 different wave lengths Weak light adaptation caused by stimulus light which was given at 2 min intervals The procedure was the same as in Fig 4 The same x and y axis criterion amplitudes and symbols as in Fig 3 Average of three subjects

ERG thresholds

A b wave of about 60 μV appeared on wave length record 499 nm at stimulus intensity of log I_s = –5 0 When monochromatic stimuli of 472 and 551 nm were used there was no b wave recordable until stimulus light of log I_s = –4 6 (or more) When more intense stimulus light was used (log I_s = –4 0 or stronger) there was also a recordable b wave on the 450 and 579 nm record (Fig 4) An a wave of 22 μV was elicited at stimulus intensity of log I_s = –2 3 on the 499 nm record Using wave lengths of 450 551 and 579 nm the a wave was not record able until the intensity of stimulus light was log I = –1 6 or stronger (Fig 4)

The oscillatory potentials were recorded distinctly at stimulus intensity of log I_s = –2 6 using wave lengths of 450 472 and 499 nm On the records of 551 and 579 nm they appeared at log I_s = –2 0 or stronger (Fig 4)

Evidently the threshold of the a- and b wave was lowest on the 499 nm record respectively The oscillatory potentials were most easily recordable when light of 472 and 499 nm was used Most prominent oscillations were re corded when maximal intensity of light of 450 472 and 499 nm was used

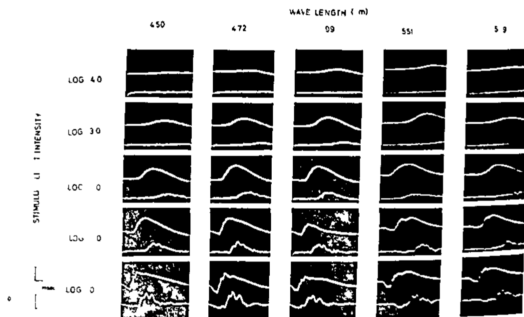


Fig 4

Threshold and relation to stimulus light intensity of the amplitude of the a and b wave and the oscillatory potentials on stimulation of lights of different wave lengths Weak light adaptation caused by stimulus light which was given at ? min intervals In other respects the procedure was similar to that described in Fig 2 Five selected wa e lengths of an equal energy spectrum at 5 different intensities are illustrated (450 472 499 551 and 579 nm)

(as well as the a wave function) from about 551 nm to about 499 nm and a change from photopic *to scotopic* shape as the light adaptation declined from strong to weak The fast components of the ERG on the other hand did not perform any significant change in the spectral sensitivity function as the light adaptation induced by light stimuli varied

Discussion

The object of this study was to evaluate the contribution of the receptor mecha nisms to the oscillatory potentials by simultaneously determining the spectral sensitivity curves for the slow (a and b wave) and rapid (oscillatory) potentials of the ERG

For the *slow potentials a transition of* maximal spectral sensitivity from the short *to the long wave* length part of the pectrum occurred as the repetition rate of light stimuli was increased The spectral sensitivity curve of the retina changed from the photopic to scotopic type i e from the characteristic shape determined mainly by the rod mechanism to the one essentially provided by cone elements However one cannot entirely exclude scotopic activity at pho topic levels and vice versa (Gouras 1966 Gouras & Link 1966 Walters 1971) and the rods continue to function over a greater range of intensities than is generally accepted (Walters & Wright 1942 Aguilar & Stiles 1954) The range *of intensities over which vision is a compound function of both rods and cones is* very extensive

The spectral sensitivity functions of the oscillations were indistinguishable in shape and locus of maximal sensitivities although the state of retinal adapta tion was changed Thus no shift of relative sensitivity was recorded with the test conditions used This is in agreement with a preliminary report of Wata nabe & Hellner (1973) which in mice ERGs demonstrated absence of the Pur kinje shift of the oscillatory potentials

There was no evidence that the oscillations should closer approximate the photopic function and be entirely a *photopic process On the* contrary under the conditions studied there was a comparatively higher *sensitivity to stimuli* of the short wave length part of the spectrum This suggests a close relation to scotopic and rod activity The results also agree with the findings of Adams & Dawson (1971) recording maximal sensitivity of the FRP of the human ERG at 500 nm during mesopic conditions

The shape of the spectral sensitivity curve of the oscillations differed from that of the slow potentials (a and b wave) It revealed two peaks at 472 nm

maximum for the oscillations The function of the oscillatory potentials did not reveal any close agreement with either the scotopic or the photopic spectral function

Consequently the spectral sensitivity functions of the slow potentials resembling the scotopic luminosity functions (CIE) differed from that of the spectral curve of the oscillations which were most sensitive to 472 nm and 551 nm stimuli

III Comparison between spectral sensitivity in strong and weak light adaptation (induced by light stimuli)

A comparison of the spectral sensitivity functions of the analysed wave forms results in Fig 6 There was a transition of maximal sensitivity of the b wave

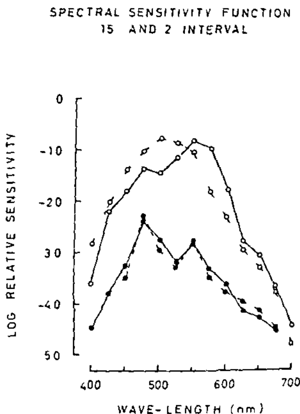


Fig 6
Comparison between the spectral sensitivity (reciprocity of threshold intensity) function of the b wave and the oscillatory potentials in response to light stimuli of 13 different wave lengths as shown in Fig 3 and 5 Black circles oscillatory potentials Open circles b wave Continuous line strong light adaptation by stimuli given at an interval of 15 sec Dashed line weak light adaptation by stimuli given at an interval of 2 min

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and 551 nm and displayed a composite curve built up from both rod and cone components This fact strongly suggests that an input from both rod and cone systems into the regeneration of the oscillatory potentials must occur This has previously been proposed in studies by Adams & Dawson (1971) Algvere & Westbeck (1972) Algvere Wachtmeister & Westbeck (1972) Algvere & Wachtmeister (1972) and Wachtmeister (1973 a b) This composite spectral curve of the oscillatory potentials is also further evidence for the oscillations being an expression for a retinal mechanism of rod and cone interaction (Nagata 1963) Such an interaction has previously been described in the monkey retina (Gouras & Link 1966 Gouras 1967) and in human psychophysical (Frumkes Sekuler Barris Reiss & Chapula 1973) and electroretinographical experiments (Walters 1971) with desaturation of monochromatic stimuli with increasing brightness

The differences in functions and performance between the slow potentials and the oscillations demonstrated clearly that the mechanism of generation of the wavelets and the slow potentials differ and is in accordance with earlier results (Algvere Wachtmeister & Westbeck 1972 Algvere & Wachtmeister 1972 Wachtmeister 1973 a b) There are also separate mechanisms which give rise to the slow and rapid components of the LRG which have been worked out in several studies (Brindley 1956 Yonemura & Hatta 1966 Werblin & Dowling 1969 Miller & Dowling 1970 Ogden 1973)

Recently Ogden (1973) suggested on the basis of microelectrode depth studies on the primate (monkey) retina that the wavelets are generated by membranes of the inner plexiform layer involving the axon terminals of the bipolar cells the processes of the amacrine cells and the dendrites of the ganglion cells He proposes the oscillations to be the result of activity in a feed back pathway – a synaptic modulation of bipolar cell axon terminals

Evidently the data provide support for the conception that the oscillatory potentials seem to reflect an interaction between the rod and cone mechanism Nevertheless further study is expected to contribute to more detailed information regarding the spectral processes of the oscillations

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CAN BENIGN LYMPHADENOSIS (LYMPHOMATOSIS) OF THE ORBIT OCCASIONALLY BE A PARANEOPLASIA?

BY

BO BAFVERSTEDT

A man with benign lymphadenosis of the orbit (published in *this* journal in 1956) remained free from symptoms after X ray treatment but subsequently died of liver metastases from a malignant epithelioma of unknown origin.
Earlier experience of benign lymphadenosis of the skin indicates that this can appear in association with and probably secondary to malignant tumours in the skin or internal organs It is postulated that the benign lymphadenosis of the orbit in the current case can have been such a paraneoplastic phenomenon

Key words exophthalmos etiology lymphoma diagnosis – lymphoma immunology – neoplasms complications – neoplasms immunology – orbital neoplasms diagnosis

In this journal Godtfredsen & Lindgren (1953) published 12 cases of benign lymphomatosis of the orbit and pointed out the probable biological identity between this and the benign lymphomatosis of the skin lymphadenosis benigna cutis described by Bafverstedt (1943) Still another orbital case was published in this journal by Bafverstedt et al (1956) who suggested the name lymphadenosis benigna orbitae for this particular kind of inflammatory tumour

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the patient approximately 27 years later But the earlier experience of benign lymphomatosis of the skin seems however in the light of modern tumour immunology to make such a connection at least hypothetical

Lymphadenosis benigna cutis should have counterparts not only in *the orbit* but also in *the thyroid the salivary glands the pancreas and the stomach intestine* probably also in *other organs* Bafverstedt (1943 1960 1968) has looked on lymphadenosis benigna cutis as a peculiar reaction to stimuli of varying kind known or unknown One can see isolated nodes develop after *insect and tickbite chronic migrating erythema* (Afzelius Lipschutz) and *trauma* It can be associated with *chronic atrophic acrodermatitis* (Herxheimer) and with *malignant tumours* of different kinds which are not necessarily localized to the skin



Fig 1
Dermatofibroma of the thigh with nodules of benign lymphadenosis at its base and in the vicinity 8 x

As we now know the development of this case and *perhaps* its cause it can be of interest to take up the discussion again

The case

Recapitulation E A labourer born 1898 had had *since 1939 a progressive right sided* exophthalmos Biopsy of a node in the lower eyelid gave cause to suspect an allergic process and the patient was treated twice with ACTH and cortisone which brought about a considerable but transient regression of the exophthalmos followed by a serious relapse and signs of exophthalmos also on the left side A new biopsy showed a picture of highly differentiated lymphoid tissue with distinct follicles and reaction centra (as in lymphadenosis benigna cutis)

X ray treatment with relatively small doses brought about quick and total freedom from symptoms which at the time of publication (1956) had lasted for 2 1/2 years

Follow up Some years after X ray treatment was concluded the patient began having neuralgia in the shoulders back and arms later on headache dizziness and coughing and finally loss of weight icterus and enlargement of the liver He died in 1966 with liver metastases of a markedly *polymorphic undifferentiated epithelial* tumour the origin of which could not be determined (complete autopsy refused) no infiltration in the sections indicating direct connection with lymphomatous tissue or lymphomatous tumour could be seen

Not during the whole period of observation from the end of the X ray treatment *until* his death did the patient show any symptoms of his earlier exophthalmos

In conclusion A slowly progressive case (14 years) of lymphadenosis benigna orbitae which after X ray treatment with a comparatively small dose became and remained symptom free for 13 years after which time the patient died with a malignant epithelioma of unknown origin

The observation in passing that ACTH cortisone treatment had a noticeable effect on the lymphomatosis is interesting

Mortada (1971) who in several papers has discussed the lymphomatous processes in the orbit treated five cases of reactive lymphocytic hyperplasia of the ocular adnexa using prolonged oral prednisolone and was able to render the patients symptom free in all cases

Comments

Nobody would probably *a priori* be willing to assume a connection between on the one hand a benign inflammatory tumour in the orbit and on the other hand a malignant epithelioma (of unknown origin) which led to the death of

(Rees 1957) might possibly belong here Experience thus indicates that a benign lymphomatosis of the skin *can* appear as *a paraneoplasia* The new tumour immunology nowadays gives us a reasonable explanation for these phenomena

Malignant tumours form substances which act as antigens and stimulate the lymphocytes to produce cytotoxins antibodies and killer cells (T lymphocytes) which fight and kill the tumour cells thus probably keeping the tumour under control and counteracting its spread It has been shown too that in the serum agents can appear blocking the cell bound immunity thus weakening or elimi nating the immune defence which could explain the sudden growth spread and metastasing of the tumour

Applied to the actual case these arguments would seem to lead to the follow ing hypothesis a small for many years subclinical malignant epithelioma gives rise to an immune reaction expressed as lymphomatosis in the orbit and finally when the immunological defence gives way there are metastases and the patient is dead

Obviously one cannot at present with an individual case prove the connection between malignant tumours and benign lymphomatosis but one should pay attention to the possibility of such combinations and not *a priori* dismiss them as coincidence

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Fig 2
Big cutaneous subcutaneous lump of benign lymphadenosis on the back (same case as in Fig 1)

Bafverstedt (1953) has described four cases in which lymphadenosis benigna cutis has been associated with malignant tumours in *the skin* (dermatofibrosarcoma protuberans and squamous cell epithelioma respectively) or in *internal organs* (cancer of the esophagus and liver respectively) The case with dermatofibrosarcoma is especially interesting since here lymphadenosis benigna cutis seemed to start at the sarcoma and from there to spread over the surface of the skin (Figs 1 & 2) In the case with liver cancer the disease started about 5 years before the manifest clinical symptoms of the liver tumour (a premonitory symptom?)

Similar combinations of lymphadenosis benigna cutis and malignant tumour have been described by Bonnet et al (1953) Azerad et al (1954) and Bolgert et al (1957) A case of benign lymphocytic infiltration of the skin + skin cancer

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AN IDEAL TEST OBJECT FOR THE TANGENT SCREEN

BY

LARS FRISEN

Light emitting diodes make appropriate test objects for the tangent screen because of their adjustability longevity and well defined stimulus proper ties A prototype wand is described that provides seven luminance levels differing by a factor of 0 315 and a uniform distribution of light within a solid angle of 40 degrees

Key words examination techniques – visual field – tangent screen – test objects

Plastic beads and paper discs are favourite test objects in tangent screen examinations of the visual field Major disadvantages with these *reflecting targets* are difficulties in defining their properties and their change in reflectivity with time It is particularly difficult to obtain test objects that allow reproducible charting of minimal central and paracentral field defects

A *recently available semiconductor* device the light emitting diode (LED) makes an interesting alternative test object LEDs are compact and inexpensive light sources with a lifetime of many years They do not require complicated

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Light emitting diodes make appropriate test objects for the tangent screen because of their adjustability, longevity and well defined stimulus properties. A prototype wand is described that provides seven luminance levels differing by a factor of 0.315 and a uniform distribution of light within a solid angle of 40 degrees.

Key words: examination techniques – visual field – tangent screen – test objects.

Plastic beads and paper discs are favourite test objects in tangent screen examinations of the visual field. Major disadvantages with these reflecting targets are difficulties in defining their properties and their change in reflectivity with time. *It is particularly difficult to obtain test objects that allow reproducible charting of minimal central and paracentral field defects.*

A recently available semiconductor device, the light emitting diode (LED), makes an interesting alternative test object. LEDs are compact and inexpensive light sources with a lifetime of many years. They do not require complicated

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Table I
Relationship between resistor values current drain and luminous output at * 10 V

Selector setting	Resistor ohms	Current mA	Relative luminous output
1	5,530	0 22	6
2	9 360	0 47	19
3	1 140	1 8	60
4	492	2 2	189
5	19	5 4	600
6	75	13	1 890
7	22	40	6 000

* measured with a United Detect r Technology 21A Power Meter

that the batteries will remain stable for many months even in a busy practice The wand can also be provided with an internal electronic battery check or an output stabilizing circuit if deemed desirable It is also possible to incorporate a variable frequency flicker device within the space available in the bandle.

Comments

Harrington (1963 1981) has advocated the use of luminous targets in tangent screen work primarily to ensure a constant target brightness everywhere on the screen This is hard to realize with reflecting targets because of the difficulties in obtaining a uniform illumination across the screen Within limits and in relation to the magnitude of intervals in commonly used sets of test objects this however cannot be a very important factor More important is the degree of uniformity of spatial distribution of light from the test object Paper discs and plastic beads both reflect more light in the direction of the light source than in any other direction and the electroluminescent chips used by Harrington also emit light with an uneven spatial distribution Thus neither type of target will have the same stimulus value peripherally on the screen and in its centre Presumably this explains why normal isopters tend to be circular on the

electronic circuits for their operation The luminous output is restricted to a limited part of the spectrum so limited that it can be considered practically monochromatic Presently available LEDs that emit light in the visible range are either green yellow or red in colour The luminance can easily and reproducibly be adjusted over a 1 000 fold range One single LED could thus replace a whole series of reflecting test objects

This paper describes a prototype tangent screen wand equipped with a light emitting diode and comments upon its properties

Construction

To ensure that the stimulus value is the same in all positions on the tangent screen the LED must have a uniform emission of light within a solid angle of at least 40° Also it must not be visible against the screen when turned off The Monsanto MV 50 a non pigmented LED of 2 mm diameter meets these requirements It has a nominal maximal luminous intensity of 2 8 mcd at 40 mA and 1 65 V This is sufficient for exploring very dense field defects The colour is a highly saturated red The spectral distribution is symmetrically centered on 660 nm the spectral half width is about 20 nm

The luminance is dependent on the current In the simplest circuit the current is regulated by means of a suitable resistor coupled in series with the LED Except for batteries and a switch no other circuit components are necessary However a single pole selector and several resistors are required to provide several luminance levels

The luminance intervals can advantageously be made equivalent to the time proven intervals used in the major series of targets in the well known Goldmann perimeter These intervals correspond to a factor of 0 315 (Goldmann 1945) A suitable combination of resistors providing seven luminance levels is given in Table I In actual use against a black tangent screen (illumination 25 lx) setting No 3 was found to be approximately equivalent to a 2 mm white plastic bead

The batteries (two 1 35 V mercury cells in series) the selector the resistors and a push button switch were housed in a 25 mm diameter acrylic handle with a length of 150 mm A 600 mm long flat black metal tube (diameter 4 mm) was also fitted to the handle The LED was recessed in a side opening at the other end of the tube with its optical axis perpendicular to the tube axis

The small current drain 1 3 mA in the most commonly used setting means

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tangent screen whereas their true (perimetric) shape is elliptical (Frisén 1970 a b) The increase in distance between target and subject with increasing eccentricity on the tangent screen is probably immaterial in this regard Neglecting peripheral ametropia of the eye the size of the retinal image will decrease at a compensating rate (Westheimer 1966) The retinal illuminance will thus stay constant provided that the spatial distribution of light from the test object is uniform

Light emitting diodes with a uniform distribution of emitted light will thus constitute much better defined targets Their adjustability and stability are additional major advantages as is the ease with which they may be made to flicker for flicker fusion frequency determinations The light rise and fall time is short enough (typically less than 1 ms) to produce accurate square wave stimuli The adjustable intensity in combination with the small visual angle subtended at the eye (the luminous area is about 1 × 1 mm) also makes LEDs appropriate for demonstrating the slit like scotomata encountered in early glaucoma and in insidious demyelinating optic neuropathy (Hoyt Frisén & Newman 1973 Frisen & Hoyt 1974) The same properties also permit a much more precise localization of the limits of field defects than do the equivalent reflecting objects of larger diameter Similarly LEDs fixed to the screen will provide excellent fixation marks

The restricted spectral range of the light is no disadvantage colour stimuli have long been held to be equivalent to achromatic targets in isopter charting This is true at least when bare perception of light regardless of colour is used as the threshold criterion However a supra threshold test of colour perception should also be included in the examination (Frisen 1973) The highly saturated colour of a red LED is ideal in this respect

The only disadvantage with LEDs is their change in brightness with a change in temperature In normal use this is not a critical factor furthermore it can be controlled by means of additional circuits for stabilizing the luminous output LEDs thus appear to be ideal test objects for tangent screen studies of the visual field

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visual sensitivity that the differential or incremental threshold of the eye changes at various adaptation levels The classical Weber Fechner law asserted that the ratio Δ I/I is constant over wide ranges of intensity if I is the intensity to which the eye is adapted and Δ I the increase in that intensity which is just perceptible The influence of wave length on the incremental threshold of the ERG has also been investigated (Hecht Peskin & Patt 1938 Biersdorf Granda & Lawson 1966 van Lith 1966 etc)

Recently the curve of incremental threshold of the oscillations of the human ERG was studied in response to white light stimuli It showed a flat range on exposure to background light below the level of adaptation at which the Purkinje shift of the b wave occurs before rising along a linear section (Wachtmeister 1973a) No differences in spectral sensitivity of the oscillatory potentials were found by studying the human ERG during a predominantly photopic or scotopic state of retinal sensitivity (Wachtmeister 1974) A double maxima of sensitivity in the blue and yellow part of the spectrum was revealed

For a more precise analysis of the sensitivity of the oscillations it was thought desirable to study the oscillations in light adaptation above the level of adaptation in which the Purkinje shift of the b wave had occurred Finding no variation of threshold to spectral lights during light adaptation in this study would then indicate the absence of a Purkinje shift of the oscillatory potentials

Apparatus and Methods

The apparatus similar to that previously employed (Wachtmeister 1974) was modified as described in Fig 1 The photostimulator (XBO 900 W/2 Osram Oriel xenon dc arc lamp) now supplied a background light as well as the previously used test light. The background light left the light source right angled to the test light beam passed by a 90 prisma a series of lenses a mirror and neutral density filters and was then combined with the stimulus beam by a mixing cube The test light beam passed as previously a suitable interposing system of lenses diaphragm neutral density and monochromatic (Schott λ max = 450 nm and 579 nm) filters but now also passed by the mixing cube which brought both beams together The energy levels of the wave lengths used were determined and calibrated using a radiation thermoelement The maximum luminance of white test light and background light was set to approximately 3×10^3 photopic cd m and 2×10^3 photopic cd/m corresponding to about 1×10 scotopic cd m and about 6×10^4 scotopic cd/m² respectively when related to the CIE (Commission Internationale l Eclairage 1957) scotopic luminosity curve Fixat

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INCREMENTAL THRESHOLDS OF THE OSCILLATORY POTENTIALS OF THE HUMAN ELECTRORETINOGRAM IN RESPONSE TO COLOURED LIGHT

BY

LILLEMOR WACHTMEISTER

On adaptation to white background light of different intensities the incremental threshold of the human ERG response was measured using monochromatic stimuli (λ max = 450 nm and 579 nm respectively)
The curve of incremental thresholds of the oscillatory potentials is approximately linear with a slope of about 0 3 using blue (λ max = 450 nm) as well as yellow (λ max = 597 nm) stimuli There was no sign of saturation i e rapid decrease in sensitivity of the oscillatory potentials on adaptation to the brightest background illumination used (2×10^5 photopic cd/m)
No Purkinje shift i e increase of sensitivity to long wave length stimulus on light adaptation was recorded The change of sensitivity of the slow potentials (a and b wave) on light adaptation to backgrounds of varying intensities differed from that of the oscillations
Thus the results were interpreted as further evidence that the oscillations are an expression of an interaction of photopic and scotopic activity The origin of the oscillatory potentials also seems to be separate from that of the slow potentials (a and b wave)

Key words electroretinography – oscillatory potentials – incremental thresholds – coloured stimuli – background light

This investigation is concerned with the spectral sensitivity of the oscillatory potentials of the human electroretinogram (LRG) in light adaptation It is known from psychophysical as well as electrophysiological investigations of

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and colour vision were normal The pupil was dilated with Mydriacyl® (Alcon lab) to more than 6 mm in diameter Surface anæsthesia was established by Novesin® (Wander) The fellow eye was occluded

The following two procedures were performed after at least 30 min of dark adaptation

I The threshold of the ERG was studied on adaptation to background illumination of white light of different intensities The wave length of stimulus light was 450 nm A series of three light stimuli of the same intensity was given After these three lights the intensity of the next series of light increased logarithmicaly There was an interval of 15 sec between each light stimulus even though the intensity of each series of three lights was varied over a range of 5 log units The procedure was repeated with background light intensities varying over a range of 6 log units The slow potentials (a and b wave) were recorded with a slow sweep speed and low amplification (0 2 mV/cm 20 msec/cm) in response to the third light stimulus The oscillatory potentials were displayed with high sweep speed and high amplification (0 1 mV/cm 10 msec/cm) in response to the third light stimulus The upper cathode ray beam presented the slow potentials and the lower beam displayed the oscillatory potentials simultaneously

II In another series of experiments the procedure was the same as described above except that the wave length of stimulus light was 579 nm

Results

The threshold of the ERG was recorded on adaptation to white background light of different intensities varying from log $I_B = -5\ 0$ to log $I_B = 0$ (Figs 2 & 4) Three consecutive light stimuli were given with 15 sec intervals and the ERG recorded in response to the third light stimulus The intensity of all three light stimuli varied from log $I_s = -5\ 0$ to log $I_s = 0$

1 Stimulus light of 450 nm wave length

A *ERG threshold* With the weakest background illumination (log $I_B = -5\ 0$) a b wave of 42 μV appeared in response to stimulus intensity of log $I_s = -3\ 0$ (Fig 2) On adaptation to maximal background light (log $I_B = 0$) the b wave was not recordable until stimulus intensity of maximal intensity (log $I_s = 0$) was used (Fig 2)

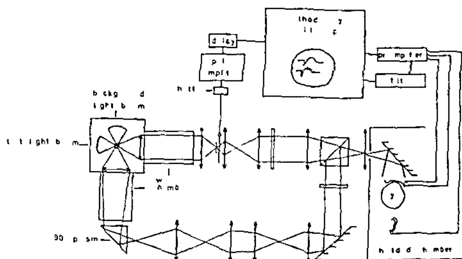


Fig 1
Diagram of optical arrangement (see text)

ion was provided for dark adaptation by a dim light spot presented to the eye for 1 sec before stimulus light appeared The colour temperature of the stimulus and background light corresponded to about 6 000°K The spectral distribution is similar to the solar spectrum The duration of light stimulus was set to 25 msec

A corneal contact lens with built in reference electrode (Lawwill & Burian 1966) was used The ground electrode was placed on the ear lobe The electric signal was amplified and displayed on a double beam cathode ray oscilloscope (Hewlett & Packard 132 A) and photographed The same calibration and band pass of the recording system was used as described previously (Algvere Wacht meister & Westbeck 1972) Measurements of amplitudes and electroretinal thresholds of a wave b wave and oscillatory potentials were performed as detailed in previous papers (Algvere Wachtmeister & Westbeck 1972 Wacht meister 1973a Wachtmeister 1974) The criterion amplitude of the slow potent ials (a and b wave) was set to 33 μV A threshold voltage of 2 5 arbitrary units (a u) was used for the oscillations All ERG s shown in the pictures were record ed from the right eye of the same subject

Procedures

The experimental findings are based on data from three young healthy subjects (two women and one man) Central visual acuity visual fields visual sensitivity

and colour vision were normal The pupil was dilated with Mydriacyl® (Alcon lab) to more than 6 mm in diameter Surface anæsthesia was established by Novesin® (Wander) The fellow eye was occluded

The following two procedures were performed after at least 30 min of dark adaptation

I The threshold of the ERG was studied on adaptation to background illumination of white light of different intensities The wave length of stimulus light was 450 nm A series of three light stimuli of the same intensity was given After these three lights the intensity of the next series of light increased logarithmicaly There was an interval of 15 sec between each light stimulus even though the intensity of each series of three lights was varied over a range of 5 log unit The procedure was repeated with background light intensities varying over a range of 6 log units The slow potentials (a and b wave) were recorded with a slow sweep speed and low amplification (0 2 mV/cm 20 msec/cm) in response to the third light stimulus The oscillatory potentials were displayed with high sweep speed and high amplification (0 1 mV/cm 10 msec/cm) in response to the third light stimulus The upper cathode ray beam presented the slow potentials and the lower beam displayed the oscillatory potentials simultaneously

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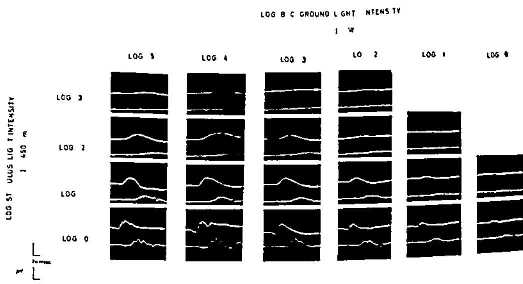


Fig 2
Threshold and relation to stimulus light ($\lambda = 450$ nm) intensity of the slow (a and b wave) and the oscillatory potentials on adaptation to background light of different intensities The background light (white) was varied over a range of 6 log units A series of three light stimuli of the same intensity was given The intensity of all three stimuli increased in a logarithmic scale There was 15 sec between each stimuli although the intensity of the stimulus varied Each ERG was displayed in a slow cathode ray sweep speed (upper trace) and simultaneously in a rapid cathode ray sweep speed (lower trace) The ERG in response to the third stimulus light in a series of three is illustrated in each picture

An a wave of about 24 μV was recorded in response to stimulus light of log $I_s = 1\ 0$ when the weakest background illumination was used (Fig 2) A distinct a wave was not elicited even when the strongest stimulus intensity was used on adaptation to background intensity of $I_B = 0$ (Fig 2)

The oscillatory potentials were recorded at stimulus intensity of about log $I_s = -1\ 0$ (Fig 2) on adaptation to the weakest background illumination On adaptation to maximal background light intensity ($I_B = 0$) the oscillatory potentials were not recordable even when maximal stimulus light intensity was used (Fig 2)

B *Incremental thresholds* The amount of stimulus intensity (Δ I) required to evoke a criterion amplitude of the slow (a and b wave) and oscillatory potentials on adaptation to background illumination of different intensities are shown in Fig 3 A criterion voltage of 33 μV was used for the a and b wave The threshold response of the oscillatory potentials was 2 5 a u (arbitrary units)

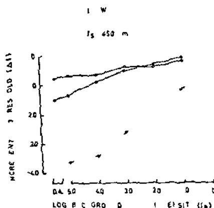


Fig 3

Incremental threshold (differential threshold) of the oscillatory potentials the a and *b wave on adaptation to background light of different intensities* The procedure was the same as in Fig 1 The incremental threshold (Δ I) is plotted against background light intensity on the x axis Average of three subjects Black circles oscillatory potentials Open circles – continuous line a wave Open circles – dashed line b wave

For the b wave the incremental threshold curve shows a decrease in sensitivity of 3 log units and rose with a fairly uniform slope which was calculated to be about 0 70 within the range of adaptation examined

The threshold of the a wave varied very little only over a range of 0 5 log units The incremental threshold curve approximated a linear function the slope of which was calculated to be about 0 12

The curve of incremental threshold for the oscillations appeared to be a linear function with a slope of about 0 35 The threshold of the oscillatory potentials diminished about 1 5 log units as the background illuminated increased

Evidently there was a difference in the slope of function between the a wave b wave and oscillatory potentials The slope of the b wave was steeper than that of the oscillatory potentials which was somewhat steeper than that of the a wave

II Stimulus light of 579 nm wave-length

A *ERG threshold* On adaptation to weak background light of log $I_B = -5 0$ a *b* wave of 57 μV was recorded in response to stimulus of log I = –3 0 (Fig 4) When maximal background illumination (log $I_B = 0$) was used a b wave of 42 μV appeared *in response to stimulation* light of log I = –0 3

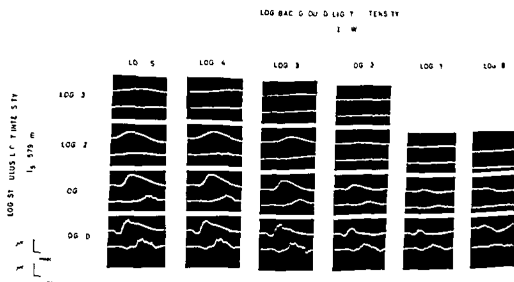


Fig 4

Threshold and relation to stimulus light intensity ($\lambda = 579$ nm) of the amplitude of the *slow (a and b wave) and the oscillatory potentials on adaptation to background light* (white) of different intensities The procedure was the same as in Fig 2 except that the wave length of the stimulus light was different

Stimulus light of log I = –1 0 was required to evoke an a wave of 40 μV on adaptation to background light of the lowest intensity (log I_B = –5 0) (Fig 4) No a wave was recorded in response to the most intense stimulus light used on adaptation to background light of strongest intensity (log I_B = 0)

B *Incremental thresholds* The constant criterion response curve of the ERG was calculated and plotted against logarithm of the adaptation illumination as described

The incremental threshold curve for the b wave rose with a fairly constant slope which was calculated to be about 0 60 There was a decrease of the threshold of about 3 log units as the adaptive light increased

The a wave *showed an approximate fit to a linear function with* a very shallow slope of about 0 07 and varied over a range of only 0 3 log units as the background light changed

The threshold of the oscillatory potentials decreased about 1 6 log units when the background illumination was augmented The incremental threshold curve rose approximately linear with a slope of about 0 32

Consequently with this procedure there was also a difference between the slope of the incremental threshold curves of the a wave b wave and the oscilla

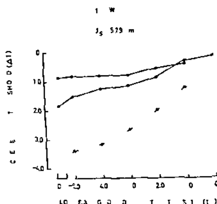


Fig 5

Incremental threshold (differential threshold) of the oscillatory potentials the a and b wave on adaptation to background light of different intensities The procedure was the same as in Fig 3 Average of three subjects The same x and y axis and symbols as in Fig 3

tory potentials The slope of the a wave was most shallow whereas that of the oscillatory potentials was less shallow The slope of the incremental threshold function of the b wave was steepest

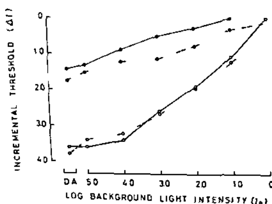


Fig 6

Comparison between the incremental threshold curve of the b wave and the oscillatory potential in response to light of 450 nm and 5 9 nm respectively as shown in Figs 3 & 5 Black circles oscillatory potentials Open circles b wave. Continuous line wave length of stimulus light 450 nm Dashed line wave length of stimulus light 5 9 nm

III Comparison between incremental threshold curves in response to light stimuli of 450 nm and 579 nm wave length

Fig 6 displays the comparative effects of colour on the incremental thresholds of the oscillatory potentials and the b wave at different light adaptation levels. The b wave curves for 450 nm and 579 nm wave length stimuli were approximately unchanged although there was a vertical shift of about 0 3 log units in maximal background illumination (log $I_B = 0$) There was a vertical separation of 0 2–0 5 log units between the 450 nm and 579 nm curves for the oscillatory potentials There was no colour effect on the slope of the functions of the oscillatory potentials The slope of the b wave function was somewhat more shallow in response to test light of 579 nm wave length in comparison to stimulation with light of 450 nm wave length

Discussion

Recently on light adaptation to light stimuli delivered at 15 sec intervals the spectral sensitivity curve of the oscillatory potentials of the human LRC was found to reach peak sensitivity at 472 nm and 551 nm and approximated neither the photopic nor the scotopic CIE luminosity curve The slow potentials (a and b wave) under these conditions revealed a spectral sensitivity curve mainly of the photopic type (Wachtmeister 1974) With light adaptation the decrease in retinal sensory as well as electrographical (b wave) sensitivity to blue stimuli is larger than for yellow i e the Purkinje shift of sensitivity within the spectrum occurs (Purkinje 1825 Wald 1945 van Lith 1966 and many others) The principle finding of the present investigation is the parallel rise of the incremental threshold for the blue (450 nm) and yellow (579 nm) light stimuli (Fig 6) No shift of sensitivity towards a longer wave length on adaptation to background light appeared i e the Purkinje shift was absent for the oscillatory potentials

An increase of sensitivity on light adaptation to yellow stimuli has been suggested as an indication of an increase of photopic activity Thus the lack of a Purkinje shift for the oscillations might be interpreted as a comparatively less active photopic system in the regeneration process of the oscillatory potentials even during adaptation to intense background illumination This is in agreement with the findings of Watanabe & Hellner (1973) that no Purkinje shift of the oscillatory potentials of the rod dominated mice retina was found although in their experimental conditions background light of less intensity was used

The incremental threshold functions of the slow potentials (a and b wave) were not appreciatively affected by wave length change The shift from an

approximately scotopic to a mainly photopic type of spectral sensitivity function had already occurred when no background light was used as the stimulus light delivered at an interval of 15 sec induced a comparatively strong light adaptation to stimulus light (Wachtmeister 1974) and the change of spectral sensitivity does not seem to be directly associated with bleaching of visual pigment (Green 1973) The background light intensity used was also above the level of adaptation at which the Purkinje shift of the b wave occurs (van Lith 1966) Thus no major difference of the spectral sensitivity of the slow potentials (a and b wave) was expected

The slope of functions of the oscillatory potentials and the b wave was approximately the same when light stimuli of 450 nm and 579 nm were used There was no essential difference in the slope of functions when stimuli of white light were employed for a shorter duration and repeated at somewhat longer intervals (Wachtmeister 1973a) For the a wave function wave length variation did not induce any change of the slope The slope of functions was much more shallow compared with the data previously reported when white light stimuli of shorter duration and longer intervals between stimuli were used This would be interpreted as a result of even less extensive temporal summation and an expression of a comparatively larger photopic activity of the a wave (Barlow 1957 1958 Biersdorf Granada & Lawson 1966) This finding would also agree with the fact that no sign of rapid decrease in sensitivity of the electric response i e saturation on exposure to background light of log $I_B = -3.0$ (around 1.8×10^3 scot td in the present study) or more was recorded As demonstrated psychophysically and electroretinographically *incremental threshold* as a function of background intensity does not saturate for cone vision (Alpern Rushton & Torri 1970 van Norren & Padmos 1973)

Thus there seems to be further evidence for an independent behaviour of the oscillatory potentials and the slow potentials (a and b wave) in the control of sensitivity of retina. The a wave (late receptor potential) reflecting receptor cell and the b wave probably generated by bipolar cell and the oscillatory potentials driven by cells in the inner plexiform layer have recently been analysed and found in intracellular experiments on the mud puppy to have different functions and ranges of operation in the process of vision (Werblin 1973) The amacrine cells probably the generators of the oscillatory potentials would then modulate the contrast sensitivity of retina (Werblin 1973) A retinal function which suggests a great ability of the neurons to operate under scotopic as well as photopic conditions This would also agree with the present findings that the oscillations seem to reflect scotopic as well as photopic activity of retina which has previously been suggested (Nagata 1962 Algvere & Wachtmeister 1972 Wachtmeister 1973b etc)

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THE FACILITY OF AQUEOUS OUTFLOW CALCULATED FROM THE RATE OF FALL IN APPLANATION PRESSURE AFTER INTRAVENOUS ACETAZOLAMIDE IN MAN

BY

OLE I NISSEN and RUD FL HOPPE

The fall in applanation pressure after intravenous acetazolamide was followed in seven patients with normal intraocular pressures and in five patients with monolateral glaucoma The experimental pressure decay curves were compared with similar curves calculated on the basis of different values for the facility of outflow of aqueous humour and on the assumption of a prompt shift in the rate of aqueous secretion from the preinjectional level to a stable acetazolamide dominated level The experimental decay curves resembled the matching calculated curves The outflow facilities read from the latter averaged 0 4 (range 0 2–1 0) in the normal eyes and 0 1 (range 0 05–0 15) in the glaucomatous eyes These results strongly indicate that the above mentioned assumptions are justified and that the method allows a simultaneous estimation of outflow facilities in the right and left eye at pressures below the usual We find that a study of the shape of the decay curves in glaucomatous eyes might give a clue to the problem of whether the hindrance to the drainage of aqueous humour in the diseased Schlemm system shows reversibility under the decompressing effect of the falling intraocular pressure

Key words glaucoma – normal eyes – applanation tonometry – acetazolamide – intraocular pressure – facility of outflow

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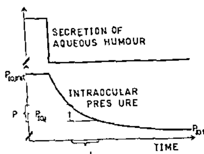


Fig 1
Hypothetical variations in the rate of secretion of chamber fluid and in intraocular pressure with acetazolamide (see text for details)

The administration af acetazolamide is known to inhibit the secretion of aqueous humour (Kolker & Hetherington 1970 Havener 1970) Immediately after the administration of the compound the rate of aqueous secretion is lower than the rate of aqueous drainage and the intraocular pressure will consequently fall (Fig 1) However due to the fall in pressure the rate of aqueous drainage will diminish and another steady state is reached where the rate of drainage equals the rate of secretion and the intraocular pressure will therefore again become stable The size of the eye will diminish due to the fall in intraocular pressure and the elastic properties of the eyeball If the duration of the change from the undisturbed rate of secretion to the rate influenced by acetazolamide is short compared to the time taken for the surplus of aqueous humour to drain from the eye the steepness of the fall in intraocular pressure should indicate the facility of the aqueous humour to leave the eye

In order to test this hypothesis experimental pressure decay curves have been compared with curves calculated from different outflow facilities on the assumption that the change in secretion is rectangular (Fig 1)

Calculations

On the assumption that Poiseuille s law is valid in the ocular outflow system for aqueous humour (the Schlemm system) the flow drained through this (F_D) is proportional to the difference between the intraocular pressure (P_{IO}) and the episcleral venous pressure (P_V) The coefficient of proportionality (flow/pressure) is called the facility of aqueous outflow (C)

$$F_D = C \ (P_{IO} - P_V) \quad (1)$$

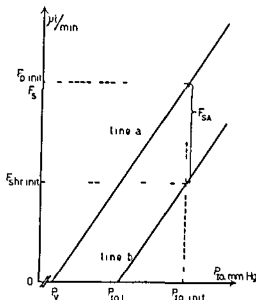


Fig 2

The flow of aqueous humour through the drainage system (F_D) and the rate of change of intraocular volume (F_{shr}) versus intraocular pressure (P_{IO}) (line *a* and *b* respectively) F_S = the flow of secretion before occurrence of effect of acetazolamide on the secretory epithelium F_{SA} = flow of secretion from the acetazolamide affected epithelium $F_{D\ init}$ and $P_{IO\ init}$ the flow through the drainage system and the intraocular pressure at the moment acetazolamide hits the secretory epithelium $P_{IO\ t}$ = the stabilized intraocular pressure determined by the acetazolamide affected rate of secretion (F_{SA}) and the facility of aqueous outflow P_V = the episcleral venous pressure (for details see Calculations)

In Fig 2 line *a* illustrates this relation (F_D versus P_{IO}) It has a slope of C and cuts the abscissa where P_{IO} equals P_V

At a stable P_{IO} the inflow of secreted aqueous humour (F_S) equals the outflow (F_D) On condition that acetazolamide will disturb this balance by provoking a fast fall in F_S to a lower and constant level (F_{SA}) without affecting either P_V or C P_{IO} will gradually fall towards a new and stable level ($P_{IO\ t}$) at which the rate of secretion again equals the rate of drainage During the pressure decay the volume of the eye diminishes the eyeball shrinks with a rate (F_{shr}) which at any P_{IO} is the rate of aqueous outflow minus the rate of aqueous inflow

$$F_{shr} = F_D - F_{SA} \qquad (2)$$

The relation between F_{shr} and P_{IO} therefore appears from Fig 2 as line *b* by substraction of F_{SA} from the ordinates of line *a* The slope of this line is also C and it cuts the abscissa in $P_{IO\ t}$ where $F_D = F_{SA}$ ($F_{shr} = 0$)

The equation for line *b* is as follows

$$F_{bt} = C\,(P_{10} - P_{10l}) \qquad (3)$$

In order to compare hypothesis and experiment the F_{bt} versus P_{10} relation should be changed to a P_{10} versus time relation According to Langham & Maumenee (1964) such conversion may be performed if the relation between intraocular volume and intraocular pressure is known Among several relations of this kind presented by different authors (see Duke Elder 1968) we chose the formula by Friedenwald (1954) which is also used in weight tonography

In a certain small pressure interval (ΔP_t) of the P_{10} versus time curve (Fig 1) the intraocular pressure decreases from $P_{10} + 1/2\ \Delta P_t$ to $P_{10} - 1/2\ \Delta P$ The average pressure of the curve segment can be regarded as P_{10} because the segment can be approximated to a straight line In this interval the eyeball shrinks by a certain volume according to Friedenwals (1954) formula

$$\Delta V_t = \frac{1}{0.0215} \log \frac{P_{10} + 1/2\ \Delta P}{P_{10} - 1/2\ \Delta P} \qquad (4)$$

where 0 0215 is the coefficient of rigidity of the normal eye (We assume that the rigidity is uninfluenced by acetazolamide) The average F_{bt} in the interval is the slope of line *b* times the difference between the average intraocular pressure in the interval and the pressure on the plateau (equation 3)

$$F_{bt} = C\ (P_{10} - P_{10l}) \qquad (5)$$

and time of the ocular shrinkage ΔV_{bt}

$$\Delta t = \frac{\Delta V_t}{F_t} = \frac{\frac{1}{0.0215} \log \frac{P_{10} + 1/2\ \Delta P}{P_{10} - 1/2\ \Delta P}}{C\ (P_{10} - P_{10l})} \qquad (6)$$

Then the construction of the hypothetical pressure decay curves (P_{10} versus time) is possible As an example we choose the curves levelling out at 12 mmHg (Fig 3)

$P_{t1} = 1?$ mmHg has been indicated as the lowest value on the ordinate of a *standard millimeter paper* (height 18 cm width 28 cm) For the first P_{10} interval we selected 31–30 mmHg (in order to utilize the full height *of* the paper) and calculated $\Delta t_{31\ 30}$ for $C = 0.1\ \mu l\ min^{-1}\ mmHg^{-1}$ according to the equation 6 (P_{10} 30 1/2 mmHg and $\Delta P = 1$ mmHg) The first point *is plotted with* 30 mmHg as the ordinate (1 mmHg = 1 cm) and $\Delta t_{31\ 30}$ as the abscissa (1 min = 4 mm) Then $\Delta t_{30\ 29}$ is calculated and added to the first the sum being the new abscissa and 29 mmHg the new ordinate

This procedure is continued until the interval $\Delta t_{13\ 12}$ is calculated and added

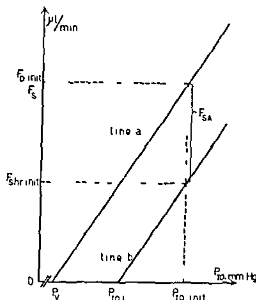


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The relation between F_{shr} and P_{IO} therefore appears from Fig 2 as line *b* by substraction of F_{SA} from the ordinates of line *a* The slope of this line is also C and it cuts the abscissa in P_{IOI} where $F_D = F_{SA}$ ($F_{shr} = 0$)

The equation for line *b* is as follows

$$F_{hr} = C \quad (P_{IO} \; P_{IO\,I}) \qquad (3)$$

In order to compare hypothesis and experiment the F_{hr} versus P_{IO} relation should be changed to a P_{IO} versus time relation According to Langham & Maumenee (19 4) such conversion may be performed if the relation between intraocular volume and intraocular pressure is known Among several relations of this kind presented by different authors (see Duke Elder 1968) we chose the formula by Friedenwald (1954) which is also used in weight tonography

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$$\Delta V_{hr} = \frac{1}{0\,0215} \log \frac{P_{IO} + 1/2 \, \Delta P}{P_{IO\,I} - 1/2 \, \Delta P} \qquad (4)$$

where 0 0215 is the coefficient of rigidity of the normal eye (We assume that the rigidity is uninfluenced by acetazolamide) The average F_{hr} in the interval is the slope of line *b* times the difference between the average intraocular pressure in the interval and the pressure on the plateau (equation 3)

$$F_{h} = C \quad (P_{IO}, P_{IO\,I}) \qquad (5)$$

and time of the ocular shrinkage ΔV_h

$$\Delta t \quad \frac{\Delta V_h}{F_h} = \frac{\frac{1}{0\,0215} \log \frac{P_{IO} + 1/2 \, \Delta P}{P_{IO} - 1/2 \, \Delta P}}{C \quad (P_{IO} - P_{IO\,I})} \qquad (6)$$

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This procedure is continued until the interval $\Delta t_{15\text{-}14}$ is calculated and added

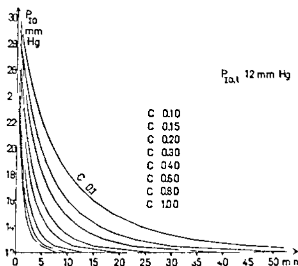


Fig 3
The curve family levelling at an intraocular pressure of 12 mmHg (for details, see Calculations)

to the preceding result and then plotted In order to obtain greater accuracy we considered intervals of 1/2 mmHg ($\triangle P_i = 1/2$ mmHg) more appropriate between 14 and 12 mmHg otherwise the procedure was the same.

Similar curves are plotted for C = 0 15 0 2 0 3 0 4 0 6 0 8 and 1 0 The result appears from Fig 3 Theoretically such "curve families should be constructed for $P_{IO\,I}$ = 8 9 etc until e g 35 mmHg in order to cover all experimental situations However in practise $P_{IO\,I}$ = 8 10 12 15 19 24 30 and 36 mmHg sufficed as neighbouring families differed only slightly At the higher $P_{IO\,I}$ values also C = 0 05 had to be calculated If the eye under study has a coefficient of ocular rigidity (k) that differs from the normal value (0 0215) the same curve families can be used after multiplication of the C value of each curve by 0 0215/k No corrections for such abnormal ocular rigidity have been applied in the present study

In different mammals including man (see Discussion) the maximal suppression of the aqueous secretion with acetazolamide seems to be approximately 50 % From Fig 2 it can be seen that if F_s is halved by acetazolamide line *b* will cut the abscissa halfway between $P_{IO\,init}$ and P_v i e

$$P_v = P_{IO\,I} - (P_{IO\,init} - P_{IO\,I}) = 2P_{IO\,I} - P_{IO\,init} \qquad (7)$$

(and furthermore as $F_{IO\,...}$ equals the rate of secretion immediately before administration of acetazolamide the latter may be calculated as

$$F_s = 2 \quad F_{s\,hr\,init} \qquad (8))$$

Material and Methods

Acetazolamide was administered intravenously generally in out patients and the intraocular pressure was followed by applanation tonometry as described by e g Kolker & Hetherington (1970) The experimental pressure decay curves were then compared with the hypothetical curves described above

The patient was placed in an office chair in front of the Haag Streit slitlamp and requested to talk or move as little as possible and to breathe naturally during the experiment Between readings the patient was allowed to move his head from the chin rest The paired pressure measurements (right left) were read with intervals of a few minutes for 15 min or more (control pressures) Through a cannula inserted in advance in an arm vein an aqueous solution of Diamox® (acetazolamide 100 mg per ml) 10 mg per kg body weight was administered during 1 min After the injection the pressure readings were resumed and continued until stable intraocular pressures were achieved

All experiments were carried out in the morning with the same instrument and the patients did not receive any instructions with respect to eating and drinking before the test

Handling of the data The applanation pressures were plotted as the ordinate (1 mmHg = 1 cm) and time as the abscissa (1 min = 4 mm) on transparent millimeter paper The pressure on the level ($P_{IO\,I}$) was read and the calculated curve family with the $P_{IO\,I}$ value closest to the experimental $P_{IO\,I}$ picked out The transparent paper was put on top of the other with the $P_{IO\,I}$ lines covering each other The tracing paper was then moved sideways so that two or three calculated curves which corresponded approximately to the experimental curves could be traced along the experimental points (see the Figures)

Patient groups The test was performed on patients with 1) normal or high normal intraocular pressure ($P_{IO\,I} < 22$–24 mmHg) 2) monolateral intraocular hypertension and 3) monolateral simple or secondary glaucoma (judged from the intraocular pressure the appearance of the papilla and the visual fields)

Three patients were given no acetazolamide the applanation pressures were followed for 3/4 of an hour to establish the stability of the intraocular pressure with undisturbed secretion of chamber fluid

Results

Description of the decay curves It appears from the Figures that in 14 of the total number of eyes (24) the intraocular pressure (P_{IO}) started to fall when the

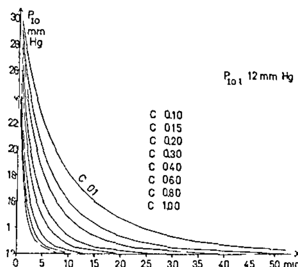


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(and furthermore as $F_{O\,init}$ equals the rate of secretion immediately before administration of acetazolamide the latter may be calculated as

$$F_S = 2 \quad F_{O\,init} \qquad (8))$$

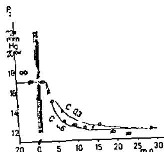


Fig 5

Patient K K F male age 75 Normal intraocular pressure Hospitalized due to bilateral cataract Optic discs deep centrally excavated not typical for glaucoma Gonioscopy open angles Visual fields normal Estimated C values = 0 4 Symbols as in Fig 4

0 15) The episcleral venous pressure in normal eyes calculated on the basis of a 50 % inhibition of aqueous secretion (equation 7) averaged 7 mmHg (range 2–12)

In one patient the arterial blood pressure was followed before and after the injection but it changed only insignificantly (see Fig 11)

No curves have been discarded as unsuccessful in this presentation One pair of curves from a patient with normal P_{10} t flatly excavated discs and scoto

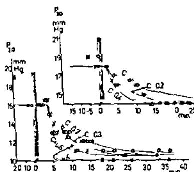


Fig 6

Patient I W male age (? Treated with prednisone (10 mg per day) for sarcoidosis (Boeck s Sarcoid) Normal intraocular pressure. Optic discs flatly excavated (nearly to the border on the right side less excavated on the left s de) Normal colour Visual fields poss bly small paracentral scotoma on both sides with 3/1000 red object 10° from the center of the Bjerrum area Otherwise normal Gonioscopy open angles The test was made twice because an unusual terrace like configuration was seen in the first curve (top) However it appeared also the second time (bottom) Estimated C values from the bottom curve = 0.35

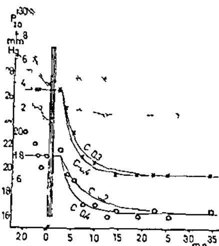


Fig 4

Patient E B male age 53 Bilateral intraocular hypertension Optic discs centrally excavated good colour Gonioscopy open angles Visual fields bilateral scotoma due to a chorioretinitis 10 years ago The fully drawn calculated curves are superposed as described under Calculations The dotted curves are pressure readings in the same patient under similar experimental conditions without acetazolamide These procedures were carried out on 2 different days Abscissa time in min Ordinate applanation pressures in mmHg The contraction of the abscissa in the control period should be noticed The bar indicates the injection time of acetazolamide (10 mg per kg body weight) x = right eye o = left eye Estimated C values = 0 3

patient sat down In the remaining eyes it was unchanged or showed a mixed pattern After approximately 10 min the pressures were fairly stable The impression was that after a few pressure readings when the examiner was familiar with the technique for each patient the readings became easier and more certain If the patients were not given acetazolamide the pressure only changed slightly (see Figs 4 7 8 and the control period in Fig 13) We considered that the initial fall in pressure could result from a fall in the episcleral venous pressure due to the patients sitting at rest

The bar indicated injection of acetazolamide 2 1 4 to 4 1/2 min (average 3 4) after the beginning of the injection the intraocular pressure began to fall steeply After varying periods of time the curves flattened and the measurements were discontinued when both pressures had been stable for some time

The decay curves from patients with normal intraocular pressure and from those with monolateral intraocular hypertension (or glaucoma) may be seen in Figs 4–15 for details see legends

The average of the estimated outflow facilities in the normal eyes (each pair counting as one) was 0 4 n = 12 (range 0 2–1 0) The outflow facilities in the monolateral hypertensive (or glaucomatous) eyes averaged 0 1 (range 0 05–

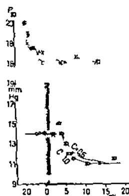


Fig 8

Patient K C male, age 68 Arterial hypertension 930/130 mmHg Intraocular pressure opt c discs and visual fields normal No gonioscopy performed Estimated C values - 1 0 The dotted curves indicate pressure readings on another patient under similar experimental conditions without acetazolamide

In human beings Becker (1959) measured an inhibition by acetazolamide of 52 % in aqueous secretion by weight tonography and 58 % in rabbits Recent *results of chamber perfusion in cats compare with these figures and further* more show an approximately parallel decrease in the transport rates of sodium ions and water Both remained at their lowest value for a period of at least 3 hours after the intravenous injection In this period the rates of aqueous secret ion were about 45 % of the control values (Oppelt 1957 Garg & Oppelt 1970) Oppelt (1967) Becker (1959) and also Friedenwald (1955) *using fluorescein*

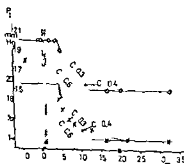


Fig 9

Patient S K J male age 49 Diabetes for 6 years treated with insulin Normal intra *ocular pressures Opt c discs* b g central excavations good colour Gonioscopy open angles Visual f elds normal Estimated C values = 0 4

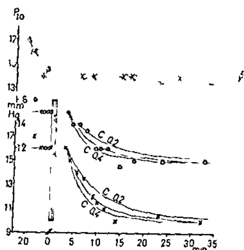


Fig 7

Patient K B male age 18 Hospitalized on suspicion of a stenosis in the left internal carotid artery Intraocular pressures optic discs and visual fields normal No gonioscopy performed The dotted curves indicate pressures under similar experimental conditions without acetazolamide Estimated C values = 0 3

mas of unknown origin in the Bjerrum areas did not fit in well with the predictions Both curves showed a terrace like configuration The test was repeated with frequent readings but the same pattern reappeared (Fig 6) The patient was calm and cooperative

Six late readings in two persons (Fig 11 – after 134 and 144 min Fig 13 – after 101 min) were discarded because the patient had been walking around the room The pressures had increased by 0–2 mmHg

Discussion

In the present experiments acetazolamide 10 mg/kg body weight was administered intravenously This dose is well within the limits recommended by the Lederle Laboratories (1962) or by Baker (1973) No complications were seen The majority of the patients could not sense the injection of the active substance however one had a cutaneous tingling during the readings and another a metallic taste In a couple of cases paresthesias were noticed some hours after the injection With reference to the studies mentioned below we hoped that this dose would suppress the rate of fluid secretion maximally during the experimental period so that postinjectional fluctuations in the plasma concentration of acetazolamide would not induce corresponding fluctuations in the ciliary production of chamber fluid The intravenous route of administration was the prime condition of a fast shift from one level of secretion to the next

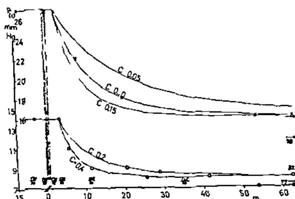


Fig 11

Patient P H ma'e age 37 R ght sided intraocular hypertension after anterior uveitis treated with steroids 2 years ago The eye is now without inflammation and treated with pilocarpine physostigmine and Eppy® eyedrops Optic discs normal on both sides without excavations Visual fields normal (1/1000 white object) Gonioscopy open angles Plenty of pigment in the trabecular network of the right side Estimated C values = 0 1 (right) and 0 35 (left) In this case the arterial blood pressures were followed and the results will appear below the figure

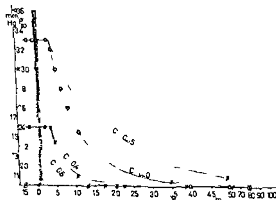


Fig 1°

I atient N F H male age 49 Left sided simple glaucoma well treated with Eppy® ey drops until a few days before the experiment. A terrace like configuration appears in the curve of the glaucomatous eye Optic d scs glaucomatous cup on the left s de and n rmal papilla cn the right Gon osc py open angles on both s des Visual fields large glaucomatous defects on the left s de (3/1000 white object) leav ng only a central and te np ral island Normal find ngs on the right side Estimated C values = 0 4 (right) and 0 05 (left)

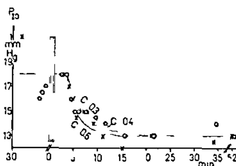


Fig 10

Patient V O J male age 54 Left sided Horner s syndrome Normal intraocular pressures Optic discs and visual fields normal No gonioscopy performed Estimated C values = 0 45

appearance time agree that the acetazolamide approximately halves the rate of water secretion this maximal response being achieved by using 10 mg per kg body weight intravenously in the cat (Oppelt 1967) and 500 mg orally in human beings (Becker 1959)

In the present experiments the pertinent and unclarified point in the interpretation of the pressure decay curves was thus Does acetazolamide induce a shift from one level of secretion (F) to the next (F) which is so prompt that the pressure decays can be accounted for on the basis of characteristics in the drainage system for chamber fluid (as sketched in Fig 1) or is the shift in secretion so slow that this factor will partially limit the rate of fall in intraocular pressure?

We had to rely on *1)* that the shape of the pressure decays resembled the theoretical curves and *2)* that the outflow facilities of calculated decay curves fitting the data of normal eyes were in reasonable accordance with facilities reached by other methods and *3)* we also wanted to test whether the method could disclose a lower outflow facility in eyes where such an impaired outflow was expected

With respect to *1)* The resemblance between the experimental and the matching calculated decay curves must be judged in the light of the inherent difficulties in estimating the pressure in the chamber fluid accurately by an external method measuring in discrete steps of 1 mmHg (applanation tonometry) and in the light of errors introduced by small incidental changes in the episcleral venous pressure or intraocular blood volume We think that there is a satisfactory agreement between the hypothetical and the experimental curves Besides it is of more interest to study the shape of the long and deep decay curves of the abnormal eye where these noise factors are of less importance

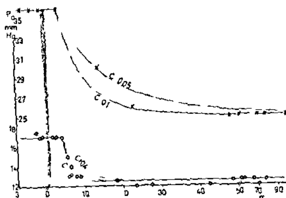


Fig 14

Patient R B male age 66 Right sided intraocular hypertension Usually treated with pilocarpine and Eppy® eyedrops however the pressure is difficult to normalize The test was performed on the untreated eye Optic discs glaucomatous cup on the right side Normal papilla on the left Gonioscopy open angles Visual fields normal Estimated C values = 0 05 (right) and 0 6 (left)

During calculations C was assumed to be unaffected by acetazolamide Opinions differ on this problem Galin & Harris (1966) measured by weight tonography an improved facility in some acetazolamide treated glaucomatous patients In early simple glaucoma Kronfeld (1962) for the most found no change in C but in some cases a fall was seen Garg & Oppelt (1970) found on an average a slightly larger recovery ($P > 0\ 07$) of inulin from their chamber perfusate for doses equal to or above 10 mg per kg of acetazolamide indicating a lowered C value however the change was regarded as being of questionable physiological significance Kupfer et al (1971) did not find any change in C in normal subjects however in some normal human eyes Becker (1959) observed a decrease in C after acetazolamide interpreted as an expression of a homeostatic mechanism tending to keep the pressure within certain limits Obviously where there is a homeostatic mechanism any method which measures outflow characteristics of the eye by imposing a pressure change inside the eye may activate this mechanism The fact that such a homeostatic mechanism is apparently triggered in some patients by the intraocular pressures depressed with acetazolamide (Becker 1959) even if the method u ed to measure the outflow facility in these patients (weight tonography) imposes a large increment of intraocular pressure due to the weight of the tonometer perhaps indicates that such baro reflex (if existing) is slow in onset and does not disturb fast working methods (as tonography and the present method) Actually in the present experiments a fast reacting feed back mecha

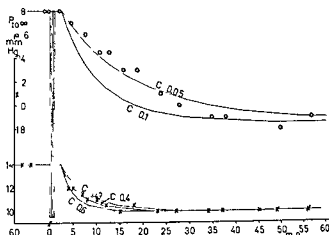


Fig 13

Patient O E male age 59 Left sided intraocular hypertension probably secondary to a heterochromic anterior uveitis The patient had been hospitalized two months before the test and a meningioma was removed Optic discs could not be inspected on the left side due to a dense cataract Right sided sequelae after papillary oedema Gonioscopy open angles on the right side The angle was open on the left side but plenty of gonio synechies were observed Visual fields this examination was not performed Estimated C values = 0 4 (right and 0 05 (left)

2) The outflow facilities in patients with normal eyes read from the matching calculated curves (average 0 4 range 0 2–1 0) are of similar magnitude as those obtained by other methods including perfusion experiments on living eyes and tonographic measurements (cf Duke Elder 1968) These give average values of 0 25–0 33 in normal eyes the higher values being found by tonography The single tonographic results range from 0 12 to 0 66 Considering that the calculating method of weight tonography also incorporates the volume indented by the Schiotz tonometer the agreement between the two different approaches is very acceptable Furthermore it should be kept in mind that the magnitude of the facility of outflow in the lower range of intraocular pressure as studied here is mainly unknown as up till now the methods have been based on increments in pressure above normal – These considerations strongly indicate that the rate of secretion drops sufficiently fast to prevent the rate of pressure decay from slowing down

3) We found that the pressure fell more slowly (C averaging 0 1 with a range of 0 05 to 0 15) in eyes where the drainage system was probably insufficient (while the normal fellow eye showed a normal decay) For comparison an outflow facility measured by weight tonography is generally considered definitely abnormal if below 0 15 Becker & Christensen (1956) found an average C value of 0 17 in simple glaucoma

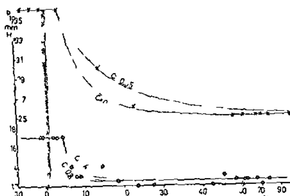


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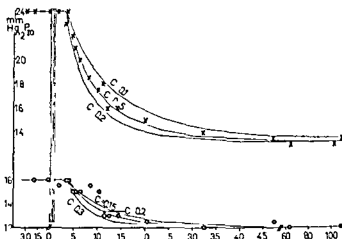


Fig 1b

Patient J P male age 38 Two years before the test the patient had a penetrating trauma of the right eye complicated by a secondary cataract and irisdialysis Since then the intraocular pressure on the right eye has been varying between 26 and 45 mmHg Optic discs normal on the left eye Inspection of the right disc was impossible Gonioscopy open angles on the left side On the right side scar tissue had partly destroyed the angle Plenty of goniosynechies were seen Visual fields this examination was not performed Estimated C values 0 15 (right) and approximately 0 2 (left)

nism decreasing the facility of outflow as a response to the acetazolamide provoked pressure fall should show up as more or less abortive pressure responses However this was not the case As mentioned earlier with a 50 % decrease in secretion the intraocular pressure should reach a point halfway between the initial intraocular pressure and the episcleral venous pressure (equation 7) Or otherwise expressed the episcleral venous pressure calculated on the basis of a 50 % decrease should be normal In normal eyes we found a range in P_v of 2–12 mmHg (average 7) which are reasonable values for sitting persons (Duke Elder 1968) In this connection it should be mentioned that P_v is not influenced by acetazolamide (Duke Elder 1968 Kupfer et al 1971)

It has been our aim to develop a method to follow outflow characteristics for aqueous humour through the range of intraocular pressures below usual This may be of interest in the eye with a diseased chamber angle where a decompression as that produced by acetazolamide may reverse a partial collapse of the Schlemm system (with respect to the collapse theory see Nesterov 1970 and Johnstone & Grant 1973) Such unfolding of the outflow channels should produce predictable changes from the curved smooth form of the calculated pressure decay

A closer discussion of this problem cannot be achieved before further experiments have been made on glaucomatous eyes

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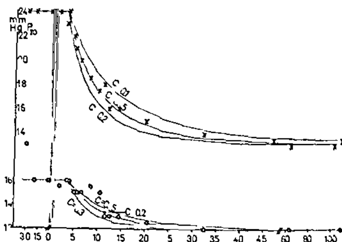


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TRANSACTIONS OF THE DANISH OPHTHALMOLOGICAL SOCIETY 1972–1973

BY

TH ROSENBERG Secretary

446th Meeting October 28 1972 in Århus (Århus Municipal Hospital)

F Kruse Hansen *Measurement of corneal thickness*

About 100 years ago Blix in Sweden performed the first optic measurement of central corneal thickness Since then many workers have done similar measurements with varying techniques

The principles of the Blix Gullstrand and von Bahr methods were reviewed Thereafter the principle of the Haag Streit pachometer was set out The side difference in corneal thickness measured by this apparatus is due to a measuring error caused by angle kappa The results obtained by the apparatus were mentioned The normal central corneal thickness is 0 520 ± 0 018 mm This is a quantity of normal distribution independent of age and sex On the other hand it has not been elucidated whether refraction affects corneal thickness There is a significant correlation between corneal thickness and intraocular tension in normal eyes Mention was made of the possible role of this factor in so called ocular hypertension Furthermore it has been demonstrated that corneal thickness is decreased in patients with retinal detachment possibly because of under developed connective tissue This hypothesis is supported by the finding of connective tissue changes in cutaneous biopsies from patients with retinal detachment

Discussion V *Dreyer N Ehlers E Goldschmidt E Gregersen P M Møller V Ohrt E Vesterdal N Willumsen*

J Nehen *Glaucoma in orbital lymphangioma*

In a boy aged 11 years monocular glaucoma occurred as a result of extraocular venous obstruction due to a retrobulbar tumour most probably a lymphangioma

The prognosis and therapeutic possibilities were discussed

Glaucoma of this pathogenesis is rare and there have been no previous reports on lymphangioma as the responsible factor

This case has been published *in extenso* as Secondary glaucoma caused by orbital lymphangioma in *Acta ophthal (Kbh)* 50 1972 495

Discussion V *Ehlers P M Moller E Vesterdal*

H E Heuer *Scanning of orbital and intraocular tumours* Publ in *Annales d'oculistique* 05 1972 983–990

Discussion *E Vesterdal N Willumsen*

V A Jensen *Simultaneous fluorescence angiography of the iris and fluorescence colour photography of the fundus*

Demonstration which will be followed by a paper

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N Ehlers *Results of human corneal grafting and tissue compatibility* Publ in *CIBA Symposium on Graft Failure* London 14 11 -17 11 1972

Discussion *P Barfoed J Edmund E Gregersen P M Moller Th Rosenberg*

447th Meeting November 17 1972 in Copenhagen (Rigshospitalet Blegdamsvej)

E Goldschmidt J Holmark & M Verdich *Treatment of lacrimation*

A follow up on all patients treated for lacrimation in the Eye Department of the Odense Hospital during the period 1 4 1968 to 1 4 1972 was reported

The material was divided into three groups considered separately

1 Treatment of lacrimation in infants
2 Dacryocystorhinostomy
3 Treatment of ruptures and strictures in the lacrimal ducts

(1) This group comprised 9 infants 9 of whom had stenosis of the lacrimal ducts on both sides Probing was done by a metal probe through the upper punctum with the assistance of a rhinologist who secured the position of the probe in the nose In a number of cases infraction of the inferior concha was performed if it was estimated to have contributed to the obstruction.

At follow up which comprised 5? of the 9 patients it was found that 40 were symptom free 7 had periodical lacrimation, whereas 2 had unchanged severe lacrimation and had derived no benefit from the probing

On the basis of these findings the following regimen was suggested Conservative treatment until the infants are 3–4 months of age In addition to local treatment by antibiotics, it is important to give instruction about expression of the lacrimal sac At

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the age of 3–4 months the infants are then subjected to probing If this does not have a lasting effect it is repeated always with the assistance of a rhinologist If three probings have not prevented a recurrence dacryocystorhinostomy is carried out when the patients are one year old

(2) By way of introduction the literature on this subject was briefly reviewed Dacryocystorhinostomy by the method of Dupuy Dutemps was emphasized as better suited than the method originally advocated in 1904 by the Italian otologist Toti

This group comprised 25 patients who had undergone dacryocystorhinostomy A total of 27 operations had been performed The results were fully satisfactory in 85 % whereas in 15 % the operation had no effect

The marked female preponderance in this group was briefly discussed It has not been possible to disclose the aetiology of the clinically manifest stenoses

(3) Of the 22 patients in this group 10 were treated in the first hours after the lacrimal canaliculi had been disrupted whilst the remaining 12 had strictures partly of traumatic origin The methods of treatmen with the Worst plastic probe or Veirs rod were described

At follow up excellent results were found in the patients who had been treated a few hours after the lacrimal canaliculi had been torn all 10 patients being symptom free

Among the others the results were poorer only 8 out of 12 having acquired proper passage to the nose

It was discussed as to how long the probe should be left in the lacrimal system and it was concluded that in cases where the canaliculi had been torn 3 weeks would be sufficient whereas in cases of later reconstructions with extensive strictures the probe would have to be left in for 3 to 6 months

Discussion *A Dreisler N Vedel Jensen*

Erik Krogh & Helmer Ring *Hepatic ophthalmia Clinical evaluation*

The basis for maintaining the term hepatic ophthalmia was evaluated in a material of non alcoholic well compensated cirrhosis None of the classical symptoms was present A few patients including women had uncharacteristic defects of colour vision within the blue yellow range The findings do not permit any conclusion but hepatic ophthalmia can be excluded from our collection of syndromes without being missed

Discussion *V Dreyer C Edmund F Godtfredsen E Goldschmidt O Nissen S Kay Andersen M Warburg*

Mette Warburg *Sex linked congenital cataract with dental malformations*

An investigation of a family with congenital sex linked cataracts microcorneas supernumerary incisors anteverted pinnae and shortened metacarpals was presented The carriers had posterior sutural opacities and cone shaped teeth The pedigree was compared with four previously reported families with sex linked cataracts one of which also presented microcorneas and with four families with sex linked microphthalmia plus a variety of somatic and mental anomalies Linkage studies could be used to demonstrate whether the diseases resulted from allelic or non allelic genes but were non contributory in the present family

To be published in *Amer J Hum Genetics* in collaboration with W L Nance D Bixler & L M Helveston

Discussion *S Ry Andersen*

E. Gregersen J Pontoppidan & E Rindziunski *Optic and drug penalization in the treatment of strabismus* Published (1974) *in extenso in Acta ophthal (Kbh) 2 60–66*

Discussion *K Bech*

E. Gregersen *Out patient after treatment of corneal grafting*

On the basis of 37 corneal graftings in the course of one year the problems concerning the ambulatory postoperative management of these patients were elucidated

In all cases the sutures were continuous nylon sutures with a few solitary knotted sutures also of nylon In most cases the solitary knotted sutures were removed 4–6 weeks after the operation The continuous nylon sutures have to be removed if and when vascular invasion occurs around the suture or if it has worked loose and has taken up a position superficially to the epithelium or when the graft is estimated to have healed firmly Thus in a few cases the continuous suture was not removed until 9 months after the operation (e g grafting done because of endothelial dystrophy with keratitis bullosa) whereas in other cases it was removed as early as 6 weeks after the grafting (e g patients with alkali corrosion in whom incipient vascular invasion showed early solidity of the scar and indicated early removal of the sutures)

The great majority of patients received fairly intensive steroid therapy (e g instillation of Ultracortenol drops (prednisolone pivalate) 6 times daily) during the first weeks after operation to reduce possible transplantation reactions and a possible tendency to goniosynechiae etc It can hardly be avoided that some patients – also those who have had grafting because of metaherpetic lesions – will need steroid therapy periodically or permanently for a number of years after transplantation in order to keep the graft free from oedema and vascular invasion as much as possible

Among the 37 grafted patients there occurred two cases of acute massive transplantation reaction with sudden severe ciliary injection and pain a dense greyish corneal oedema with keratic precipitates on the posterior surface of the cornea and visual impairment to hand movements On immediate energetic local and systemic steroid therapy the visual acuity was restored to 6 9 in one of the cases but only to 6 24 in the other

For geographic reasons the long lasting ambulatory postoperative management of the grafted patients is largely in the hands of the patients own ophthalmologists In this connection it must be emphasized that a favourable permanent result depends just as much upon the ambulatory postoperative control and treatment as upon the operation itself

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445th Meeting November 17 1972 in Copenhagen
(Rigshospitalet Blegdamsvej)
Extraordinary General Assembly

Chairman *P M Møller*

It was resolved that foreign ophthalmologists should be admitted as non paying extraordinary members of the Danish Ophthalmological Society

Discussion about the second amendment to the report on Danish ophthalmological health care

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449th Meeting December 9 1972 in Copenhagen (Rigshospitalet Blegdamsvej)

Clinical Pathological Conference

Arranged by the Institute of Ophthalmic Pathology University of Copenhagen with

S Ry Andersen and O A Jensen in the chair

Mette Warburg showed her film "*Blindisms*

450th Meeting February 17 1973 in Copenhagen (Rigshospitalet Blegdamsvej)

H Fledelius *Ophthalmic use of diagnostic ultrasound*

The subject was reviewed and a 6 year material from the Eye Clinic Rigshospitalet Copenhagen was presented A total of 1 340 examinations had been performed 537 being oculometric measurements and 808 diagnostic ultra onographies Indications results and reliability were discussed (To be published *in extenso* in *Ugeskrift for Læger)*

Discussion *E Gregersen S Ry Andersen J Edmund*

Erik Krogh *Clinical electro oculography*

Basic factors as well as the technique of measurement and electrotechnical factors in the indirect registration of the corneo fundal potential were described This was followed by a brief discussion on the applicability of the method and its possible diagnostic value in clinical practice

E Godtfredsen *Is the intraocular tension under pineal hormonal control?*

The normal and patho physiology of intraocular tension is a constant challenge to all ophthalmologists

Much is known about how and when the intraocular tension varies the phasic cyclic diurnal or circadian changes The last term is derived from the Latin *circa diem* around the day Why do these changes happen? In this respect very little is known Here modern pinealogy may be of assistance to ophthalmology Research during the last 10 years has established the pineal body also called the epiphysis cerebri as a biologi

cal clock a neuro endocrine transducer responsible for many of our circadian rhythmic variables such as plasma cortisol *serum iron* etc. This motivates a short outline of the natural history of the pineal body from the day of Réne Descartes to modern neuro biochemistry A new hormone melatonin which is a serotonin derivative has been found to be produced in the pineal body It acts as an inhibitor of the hypothalamic pituitary hormone system The production and release of melatonin are directed by light impulses from the retina transmitted via the sympathetic innervation to the pineal body When light is on viz in the daytime melatonin synthesis is off This means that the inhibition of the hypothalamus ceases and hormonal *hypothalamic activity goes on* viz stimulat ion This is the neuro endocrine background for our circadian rhythm maybe also in the eye but the proof must be left to further investigations

D i s c u s s i o n *M Warburg S Ry Andersen E Goldschmidt Th Rosenberg*

401st Meeting March 16 1973 at Lund
(Joint Meeting with the Ophthalmological Society of Southern Sweden held at the Eye Clinic of the University Hospital of Lund)

B E h i n g e r *Amino acids as retinal neurotransmitters*

For a long time it has been evident that as yet we know only a few of the neuro transmitter substances which must be present in the CNS Most recently certain amino acids mainly glycine and γ aminobutyric acid (GABA) are more than ever suspected of being inhibitory neurotransmitters also in the retina They occur in a high concentrat i n in the ret na and they have displayed distinct inhibitory effects in electro physio l gical experiments

We ha e demonstr ted that very active uptake of glycine takes place in the retina, and from other laboratories a sim lar uptake of GABA has been *reported*. With the aid of autoradiography we have been able to demonstrate that this uptake is predomi nantly caused by certain amacrine cells where the amino acid is stored in a protected way so that it is not metabolized Amino acids which are not suspected of being neuro transm tters (about twenty have been tested) are taken up in a different diffuse and n *specific* way The *amino acids* glutamic acid asparagine acid and taurine, have be n suggested as exc tatory neurotransmitters Their uptake (exclusively to glia cells) is f a third type ea ily distinguished from the other two

K netic stud es of the uptake of among others glyc ne and GABA indicate that they ay act as inhibitory ret nal neurotransm tters in amacrine cells

D i s c u s s i o n *Not ttended by the repo ter*

O H o l m *Eighteen mo ths experience of a reading device for the totally blind in Scandina ia*

Publ in an extended form in *Sven k Lakartidning* Aug 1973

D i s c u s s i o n *I Dreyer H Skydsgaard C E T Krakau*

C. L. T. Krakau and K. Wilke: *Measurements of the episcleral venous pressure by means of an air jet*

Publ. (1973) *Acta ophthal. (Kbh.)* 51, 185–196.

Discussion: *B. Ehinger*

G. Stigmar: *Bagolini's red glass test as an aid in diagnosing strabismus*

Bagolini's red glass test consists of a series of red glasses with gradually decreasing transmission. The patient is made to look at a small light source while successively ever darker red glasses are passed before his eyes. It is then recorded at which glass diplopia occurs. By this successive dissociation of binocular vision it is possible to get an idea of the degree of compensatory fusion in heterophorias. Patients having symptoms caused by heterophoria experience diplopia even at a relatively slight dissociation. With a minor modification the method is applicable also for determining the subjective near point in convergence insufficiency.

In the presence of suppression scotoma some impression of the depth of the scotoma may be gained by placing the glasses in front of the dominant eye. For instance in strabismus with harmonious ARC only a slight inhibition of the dominant eye is usually required to induce displopia.

Bagolini's red glass test is a simple one but has a wide range of applicability and it rapidly affords information of diagnostic as well as therapeutic value. It is recommended for increased use in orthoptic examinations.

Discussion: *E. Gregersen, C. E. T. Krakau*

M. S. Norn: *Scleral plaques and preplaques*

Publ. (1974) *Acta ophthal. (Kbh.)* 52, 96–106.

Discussion: *J. Edmund, H. Bynke, V. Dreyer, C. E. T. Krakau*

H. Bynke & K. Wilke: *Comparison of two different models of the oculosphygmograph*

In principle ophthalmological methods can diagnose only those cases of unilateral carotid stenosis in which the lumen is greatly narrowed. This is a disadvantage as mild stenoses may also give rise to clinical symptoms and these are the very cases most accessible to surgical treatment.

Previously a piezo-electric instrument, the oculosphygmograph, was presented in two different models (Bynke & Krakau: *Ophthalmologica* 1964; Bynke & Öhman: *Acta ophthal. (Kbh.)* 1970). The original model (Model 1) weighs 20 g, the more recent one (Model 2) 10 g. A preliminary study indicated that in cases of carotid stenosis the lighter instrument afforded a greater side difference in amplitude than did the heavier instrument. This might therefore afford increased possibilities for diagnosing mild stenoses.

Now a detailed comparison of the two models has been performed. To make the comparison more reliable, Model 2 was also tested with an extra weight of 10 g (Model 2 a).

In 33 healthy subjects the standard deviation of the mean of the side difference was the same for the different models. Application of Fischer's F test to the standard deviations for Models 2 and 2 a revealed no significant difference ($P > 0.05$).

In 25 cases of unilateral carotid stenosis the mean difference between the sides was greater with the lighter instrument (25 %) than with the heavier one (18 %) as had been indicated by the preliminary investigations However owing to a marked scatter of the measurements these differences were not significant Thus a comparison of Model 1 with Model 2 resulted in $P > 0.05$ between Model 2 and Model 2 a in $0.05 > P > 0.025$ (t test paired observations)

In other words we must conclude that the lighter instrument does not afford greater possibilities than the heavier instrument for diagnosing carotid stenosis

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452nd Meeting April 27 1973 in Århus (Scanticon)

Bjerrum Memorial Lecture H G Scheie Philadelphia *The management of congenital cataract*

453rd Meeting April 27 1973 in Århus (Scanticon)

D Davanger Oslo *Congenital cataract a material*

A material of 254 cases of congenital cataract treated during the years 1940–1972 had been analysed Stratification familial 25 % history of rubella 10 % zonular cataract 35 % with the same percentage in familial and non familial cataract excl rubella Unilateral cataract 20 % mainly belonging to the group of non zonular non familial cataract

Male female ratio total 1 4? in zonular cataract 2 20 in unilateral cataract 0 6?

Pathology during pregnancy birth in 36 % Other abnormalities in 30 % most frequent in rubella cataract (81) more frequent in non familial cataract excl rubella (3 5) than in familial cataract (21 %) The most common abnormalities were oligophrenia cardiac malformations epilepsy deafness cerebral palsy microcephalia 60 of the rubella cases were born in the first quarter of the year

Zonular cataract had the most favourable visual prognosis rubella cataract the poorest Patients with a poor preoperative vision generally had a poor postoperative visual acuity All patients with unilateral cataract had *poor postoperative visual acuity* Postoperative amblyopia was assessed

Postoperative complications glaucoma ?4 % endophthalmitis 1 2 % retinal detachment 1 6

J Edmund & H H Seedorff *Retinal detachment in the aphakic eye*

In a collected material of 2191 cases of retinal detachment two different groups were studied to explore the clinical features of aphakic retinal detachment

Although the number of aphakic detachment is increasing the occurrence of detach

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4,2nd Meeting April 2, 19,3 in Århus (Scanticon)

Bjerrum Memorial Lecture H G Scheie Philadelphia *The management of congenital cataract*

4,3rd Meeting April 27 19,3 in Århus (Scanticon)

D Davanger Oslo *Congenital cataract a material*

A material of 54 cases of congenital cataract, treated during the years 1940–1979 had been analysed. Stratification familial 25•/•, history of rubella 10•/• zonular cataract 35•/• with the same percentage in familial and non familial cataract excl rubella. Unilateral cataract 20 mainly belonging to the group of non zonular non familial cataract.

Male female ratio total 1 4 in zonular cataract 2 ?0 in unilateral cataract 0 62

Pathology during pregnancy birth in 56•/• Other abnormalities in 56•/• most frequent in rubella cataract (81) more frequent in non familial cataract excl rubella (55 5•) than in familial cataract (?1•) The most common abnormalities were oligophrenia cardiac malformations epilepsy deafness cerebral palsy microcephalia (•) of the rubella cases were born in the first quarter of the year

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Post operative complications glaucoma 4 • endophthalmitis 1 2 • retinal detachment 1 6

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In a collected material of 191 cases of retinal detachment two different groups were studied to explore the clinical features of aphakic retinal detachment.

Although the number of aphakic detachment is increasing the occurrence of detach

ment in the aphakic eye is not increasing The real increase was found in the number of aphakic patients

Somewhat surprisingly the incidence of aphakic detachments originating from congenital cataracts was exactly the same in the two groups to some extent contradicting the opinion that all aphakic eyes will sooner or later exhibit retinal detachment

Although certain clinical features distinguish aphakic detachment following congenital cataract from the others the reattachment rate was strikingly uniform

The importance of the surgical procedure in the cataract operation could not be appraised from this series The low reattachment rate following needling and extracapsular extraction is probably due to difficulty in the examination and surgery of the detachment rather than to the cataract operation

The surgical procedure of choice in aphakic retinal detachment is encircling probably quite often combined with injection of hyaluronic acid into the vitreous

The occurrence of myopia before cataract extraction in the aphakic detachments was found to be lower then among phakic detachments This seems to indicate the development of a pathological state of the peripheral choroid retina or vitreous alongside with the cataract and emphasizes a predisposition in the cataractous eye as being the fundamental primary factor in the occurrence of retinal detachment in the aphakic eye

The most dangerous type of aphakia with respect to detachment appears following cataract extraction of a juvenile cataract in an excessively myopic eye The high risk of retinal detachment and the relatively poor results of reattachment should be borne in mind when contemplating extraction in this type of patient

While the low incidence of finding a tear among the congenital and complicated aphakics is explicable the inability to discover a tear in a quarter of the cases in the juvenile and senile groups is difficult to explain even after considering the differences in the interpretation of a tear The possibility of a different pathogenesis of retinal detachment in the aphakic eye was mentioned

Harold G Scheie (guest lecturer) *Cataract extraction after filtering operation*

TRANSACTIONS OF THE SWEDISH OPHTHALMOLOGICAL SOCIETY 1973

EDITED BY

BJÖRN SVEDBERGH EDITOR

Meeting in Stockholm March 24 1973

S E Nilsson & B Knave *A new method of clinical EPC*

The present paper describes a new method developed for d c registration of the human ERG Matched calomel half cells were used as recording and reference electrodes They were connected by means of saline bridges in agar filled polyethylene tubes to a scleral contact lens and to a plastic chamber placed on the forehead The electrodes were connected to the differential inputs of a low drift d c amplifier The potentials were low pass filtered (0 Hz cut off 18dB/octave) and fed into a Hewlett Packard signal analyzer 5480 S where the responses were averaged The noise level f the electrode system was 5-10 V and the d c drift 10-15 μ V/h A 150 Watt xenon lamp with an approximately flat spectral emission curve within the visible part of the spectrum was used for l ght stimulation. The l ght intensity was changed by means of neutral density filters A Zeiss electromagnetic shutter was used to control the duration of stimulus The stimulus light was directed to the patient s eyes through a Y shaped qua tz fiber optic attached to an adjustable spectacle frame. The new method made it pos ble to in est gate retinal functions that have not earlier been studied clinically e g the r t nal re ponses below the b wave threshold the c wave (reflecting the activity of the pigment ep thelial cells) and other slow potentials after cessation of the l ght stimulus It s suggested that this method may make it possible to give an earlier diagnosis f retinal and pigment epithelial disorders induced by drugs or caused by other factors.

(This inve tigation was supported by grants from the Swedish Medical Research Council Nos. 1 X 34 and 04X 3119)

G Boman *Melanin affinity of rifampicin studied by autoradiography*

The distribution of ^{14}C labelled rifampicin in mice has been studied by whole body autoradiography according to Sven Ullberg In albino mice only faint radioactivity could be seen in the eye However in pigmented mice strong radioactivity was observed in the uveal tract and the skin even 4 days after an i v injection. This difference is interpreted as evidence for melanin affinity No certain ocular side effects have been reported in patients on long term treatment with rifampicin

B Calissendorff B Knave H E Persson & S E. G Nilsson *An electrophysiological study on the effects of a new antituberculous drug rifampicin on retinal functions*

In the present paper selective effects were reported on the c wave of the sheep ERG following i v injections of rifampicin At doses of 20 and 40 mg/kg bodyweight the normal oscillations of the c wave amplitude were found to increase in amplitude without any concomitant changes in the a- and b waves This effect was interpreted as a functional correlative to the autoradiographical findings reported by Boman

G Stigmar *Bagolini s red glass strip as a diagnostic instrument in squint*

E Lindstedt *The vision aids department at Uppsala A presentation*

A Alm *Vasodilatory drugs and retinal blood flow*

(Based on the article Effects of norepinephrine, angiotensin dihydroergotamine papaverine isoproterenol histamine nicotinic acid and xanthinol nicotinate on retinal oxygen tension in cats *Acta ophthal (Kbh) 50* (1972) 707–719)

Symposium Dyslexia in children – visual disturbances

Moderator A Holmberg Participants G Aurell G von Bahr T Hedqvist K G Nyman & G Stigmar

Meeting in Stockholm December 1 1973

SYMPOSIUM – SOFT CONTACT LENSES

Moderator \ Holmberg

I articipants

B Wulfing *Properties of and problems with s(ft contact lenses*

M Rydberg *Soft contact lenses as treatment of corneal diseases*

E. Dahlstedt *Soft contact lenses in myofia*

B Wulfing *Soft contact lenses in aphakia*

VARIA

T Jerndal & \ I rssea *Results of the trabeculectomy operation in exfoliation glaucoma*

I A Cranstrom U Axelsson J Bengtsson & \ Holmberg
8–0 Eth icon chromic collagen suture in cataract surgery

Fifty eyes operated with 8–0 Ethicon chromic collagen have been compared with 50 eyes operated with 8–0 silk suture as regards postoperative complications All operations were performed with a limbus based conjunctival flap

Three wound ruptures occurred in the collagen group The ruptures were caused by direct trauma in two of the three cases In the group operated with silk rupture occurr ed in ne eye and n this case there was no known trauma In seven eyes in the collagen group one or several sutures eroded through the conjunctival flap while the correspond ng figure was 11 in the gr up operated with silk It was notable that none of the seven patients in the collagen group had any discomfort On the other hand m re than half of the patients with silk sutures through the flap had so much discomfort that it was necessary to rem e the sutures We have not seen any increased postopera t ve inflammati n of collagen compared with the silk suture On the contrary we have the i npres ion that s lk is more irritable to the eye than collagen.

L Frisén *Fundoscop c evaluation of the retinal nerve fiber layer*

Ba d on the foll wing articles

H yt W F Frisén L & Newman, N M Fundoscopy of nerve fiber layer defects in glaucom a *In est Of t al* (Nov 19 3) Frisén L & Hoyt W F Insidious atrophy of ret nal ner e fiber in multiple sclerosis Fundoscopic identification in patients with and w th ut v sual complaints Arch. Ophthal in press

M Lundstrom *Defects in the reti al nerve fiber layer in gla coma*

C Lennerstrand *Proprioception in ocular muscles*

B Wulfing *A case of ocul r muscle paresis cured with manipulation*

L. O. Almér, M. Pandolfi & S. Österlin: *Fibrinolysis in diabetes mellitus with special reference to diabetic retinopathy*

The spontaneous fibrinolytic activity, the fibrinolytic response to standardized venous occlusion of the arms and the fibrinolytic activity of venous biopsies were studied in 135 patients with diabetes mellitus (DM).

The mean spontaneous fibrinolytic activity was lower in patients with DM than that of 153 sex- and age-matched non-diabetics. The difference was not significant.

The fibrinolytic response to venous occlusion of the arms was significantly lower ($P < 0.001$) in patients with DM than in the control group. No difference was found between patients with and without retinopathy. However, patients with retinopathy showed a progressively decreasing fibrinolytic response with the duration of the disease, while in patients without retinopathy the fibrinolytic response progressively increased and was almost normal 10 to 20 years after the onset of DM.

The fibrinolytic activity of the vessel wall was abnormally decreased in 21% of the diabetic patients; no difference was seen between patients with and without retinopathy.

It is concluded that patients with DM have, as a group, a less active fibrinolytic system. This change may contribute to the fibrin deposition in the vessel wall and to the occurrence of the vascular complications often seen in DM.

B. Calissendorff: *Smoking and night vision*

A. Hedin: *Pupillography in congenital colour vision deficiencies*

(To be published in Proc. Second Symposium of the International Research Group on Colour Vision Deficiencies, Edinburgh, June, 1973)

L. Frisén: *Visual handicap and the Pulfrich phenomenon*

(Based on the article: Frisén, L., Hoyt, W. F., Bird, A. C. & Weale, R. A. (1973): Diagnostic uses of the Pulfrich phenomenon. Lancet *i*, 385–386)

A. Öhrström & M. Pandolfi: *Clinical use of beta blockers in increased intraocular pressure*

Propanolol has been given orally in doses of 40–500 mg daily (generally 80–160 mg) to 22 patients with increased intraocular pressure (IOP) after conventional antiglaucomatous therapy had failed. A satisfactory (IOP $\leq$ 22 mmHg) or fairly satisfactory (IOP $\leq$ 26 mmHg) decrease of the IOP was obtained in nine cases with wide angle glaucoma, one with narrow angle glaucoma and one with secondary glaucoma. The follow-up period ranged from 3 to 26 months. The side effects observed were generally mild and in only one case was it necessary to discontinue the therapy. The side effects consisted mainly of insomnia and diarrhoea. According to our experience it is worth while trying propanolol when traditional antiglaucomatous therapy has failed.

JUDICIA DE NOVIS LIBRIS

Shearn Martin A Sjogren s Syndrome W B Saunders Phil Lond Toronto 1971 62 pages Price D kr 108

This monograph of Sjogren s syndrome is written by an Assistant Professor of Medicine University of California It is based on detailed investigations of 80 patients and a multidisciplinary study of literature including 549 references

Chapters deal with ocular aspects Schirmers test has only limited value rose bengal vital staining is specific for the syndrome

Electrocoagulation of the lacrymal puncta is a doubtful therapeutic measure

The many rheumatologic rhinologic, oral bronchial gastric odontologic nephropatic endocrinolog c and onchologic aspects of the disease are dealt with

Blind biopsy of buccal salivary glands is an informative simple and safe procedure it should be done before considering biopsy of salivary or lacrymal glands

Sialography and salivary gland scintigraphy fluorescens tests (antibodies to salivary duct and thyroidgland cell cytoplasm) are dealt with in detail

The poss ble relation between Sjogrens syndrome and other diseases is discussed (rheumatoid arthritis lupus erythematosus scleroderma, arteritis Hashimotos thyroiditis chronic hepatobiliary diseases macroglobulinemia lymphoma and other malignancies)

A mouse model of Sjogrens syndrome is described

The frontispiece of the book is a photograph of the Swedish ophthalmologist Henrik Sj gren (J nkoping now Lund) who gave his name and genius to this interesting syndrome

M S Norn

VARIA

The Third International Orthoptic Congress

will be held n B ston, Massachusetts U.S.A on July 1st nd and 3rd 19 5 *organized* by the Internation l Orthoptic Association in collaboration with the American Associa ti n of Certif ed Orthoptists *Information can be obtained* from the Congress Office 6 Beac n Stre t Suite 6?0 Bost n, Massachusetts 0 108 U.S.A

Those intere t d in present g *papers and films* are asked to write to the Chairman f the Sci ntific Committ e Miss J *Mein, at the* Orthoptic Department The Hallam sh re H sp t l Glo p R ad Sheffield S10 ?JF United *Kingdom*

L O Almer M Pandolfi & S Österlin *Fibrinolysis in diabetes mellitus with special reference to diabetic retinopathy*

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University Eye Hospital Oulu Finland
Head Prof Henrik Forsius

PSEUDOEXFOLIATION OF THE LENS CAPSULE AND DEPTH OF ANTERIOR CHAMBER IN NORTHERN ICELAND

BY

H FORSIUS K SVEINSSON E. ALS and H LUUKKA

A study on the occurrence of pseudoexfoliatio lentis was performed on an Icelandic population consisting of 634 persons of all age groups The frequency was high 3 16 % at 40 49 years 4 ?9 % at 50 59 years 13.56 % at 60 69 years and 43 % at the age of more than 0 years In addition two persons less than 40 years of age had pseudoexfoliatio lentis
Depth of the anterior chamber did not significantly deviate from the Scandinavian comparison material

Key words pseudoexfoliatio lentis – fibrillopathia epitheliocapsularis – capsular glaucoma – glaucoma simplex – eye pressure – anterior chamber depth – Iceland

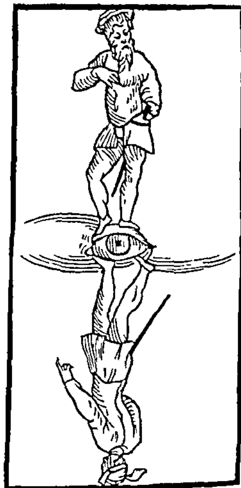
The Nordic Humanecological Research Group organized an expedition to northern Iceland in the summer of 1972 to the town of Husavik with 2 000 inhabitants The study includes genetics anthropology *physiology etc* and belongs to the Man and Biosphere series of projects supported by Unesco Four ophthalmologists participated in the field work and performed on the popula

Supported by grants from Nordic Cultural Foundation and the Finnish Man and Biosphere Committee

Received November ?6 19?3

METTE WARBURG

Diagnosis of Metabolic Eye Diseases



The book provides a s rvey of such oc lar d sorders for wh ch a metabol c bas s has been establ shed and ind cates the rele ant n est gat ons that should be pe formed to establ sh the d agnos s

The man al s p mar ly tended fo ophthal molog sts to whom pat ents are sent for e aluat on f om paed atr c neu olog cal ge et c a d metabol c depa tments and for opthalmolog sts wo k g n out pat ents cl n cs

The te t comp ses 67 d seases p esented acco d ng to the seq ence of a ro t ne ophthalm c exam nation start ng w th nspec t on of the eye as a whole p ogress g th o gh the sk n of the eyel ds the cor ea s le s v t eo s and et a For each d sease the p nc pal oc lar s gns general s gns metabol c error and laboratory f d gs n blood r ne central nervo s system sk n and other b opsy spec me s are co c sely p ese ted The genet cs and the f nd ngs in the ca r ers as ell as the treatment f ava lable are noted A sho t b bl og aphy comp s ng pa t c la ly recent art cles and survey art cles follow each tem

112 pages DK 115,00 ($16.25, £6.40, DM 56.00)



MUNKSGAARD
COPENHAGEN DENMARK

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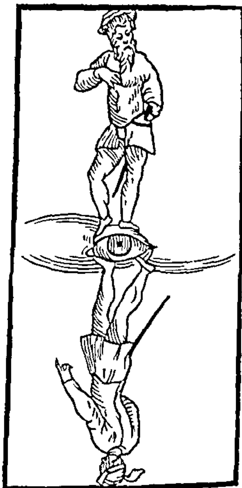
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11 pages, D kr 115.00 ($ 16.25, £ 6.40, DM 56.00)



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tion a study of the eyes including total refraction colour sense, measurements of the corneal refraction thickness and diameter as well as climatological changes in the eyes

Because Iceland has a very high frequency of open angle glaucoma while the frequency of narrow angle glaucoma is low the study programme consisted of examination of depth of the anterior chamber eye pressure and frequency of pseudoexfoliatio lentis (fibrillopathia epitheliocapsularis) The results are compared with Scandinavian population investigations

Skulason (1933) estimates that 1 % of the entire population in Iceland has glaucoma According to Björnsson (1955) 52 % of blindness in Iceland in 1950 was caused by glaucoma The blindness rate was 300 per 100 000 which is three times higher than in other Scandinavian countries In the age group > 70 years 10 1 % have glaucoma and in the age group 60 69 years 6 4 % (Bjornsson 1964) On the other hand narrow angle glaucoma is rare Sveinsson (1959) recorded 1 561 glaucoma patients in Iceland in a total population of 170 000 or 0 92 % Fifteen had secondary glaucoma two had hydrophthalmus and the rest (1 544) had primary glaucoma 90 29 % had open angle glaucoma The frequency of acute glaucoma was only 1 81 % and that of chronic inflam matory glaucoma 7 9 % Sveinsson showed that the glaucoma was hereditary in at least 45 % of all cases These figures are very high compared with the numbers shown for treated cases in other parts of Scandinavia *Westerlund* (1947) estimates the frequency of glaucoma to be 0 055 % for the total popula tion in Denmark and Forsius et al (1973) give the figure 0 346 % which is based on the number of all glaucoma cases in Finland receiving free medical care The figure for persons 65 74 years old is 3 44 % and 2 86 % for persons > 75 years

Figures found in systematic population investigations are of course higher since many glaucoma cases have not been detected and essentially vary accord ing to district and the criteria for diagnosis Thus statistics from a city in Sweden showed 4 4 % of persons > 40 years of age with ocular hypertension (Stromberg 1962) However the number of cases which led to damage in the ocular nerves was definitely lower

Koskenoja and Orma (1955) found glaucoma in 4 6 % of persons over 64 years old in Finland These Scandinavian records correspond largely to the figures from Caucasian populations in other parts of the world where the fre quency of 0 8 5 % is recorded in older age groups depending on the criteria for glaucoma

According to statistics from Oulu University Eye Hospital 14 9 % of all glaucomas in the northern part of Finland are the narrow angle type In this area 0 345 % medically treated glaucomas in the total population are registered

Figures quoted in the literature of pseudoexfoliatio lentis concerning its geographical incidence among the normal population range from 0 1 % (Egypt Maghraby 1937) to 48 % (Russia Hoorgina 1929) Aasved 1969 compared population groups in Norway (Bergen) England (Birmingham) and Germany (Bonn) and found no statistically significant differences between these localities in people over 60 years old The frequency found among men was 3 3 % 2 7 % and 7 7 % respectively Among women the incidence was 7 4 % 4 8 % and 4 0 % respectively Bartholomew (1973) found 11 3 % exfoliation above 70 years of age among the Bantu in South Africa The incidence among glaucoma patients was 19 %

Pseudoexfoliation of the lens capsule was noted by Bjornson 1964 but has not been systematically investigated in Iceland

Own Investigations

In a total of 634 persons of all age groups and both sexes were investigated The genealogist Indridason was instructed to avoid calling on near relatives except in the oldest age groups where we tried to get as complete a sampling as possible The distribution in different age groups was as follows in (Table I) The eye pressure was measured with Schiotz standard tonometer in all patients > 40 years old All patients of 45 years or older were studied after dilatation of the pupil with 10 % Metaoksedrin (Star) and Tropicamide (Mydriat Orion) in the biomicroscope for examination of pseudoexfoliation of the lens capsule and liberation of iris pigment

Frequency of pseudoexfoliation

In Table II several populations studied by our group are compared Skolt Lapps Finns in Oulu and Icelanders have about the same high frequency of

Table I
Ophthalmological investigation in Husavik Iceland
Distribution of population in different age groups

Age	0–9 49	9 5–14 49	14 5–19 49	19 5–50 49	50.5–59 49	59 5–69 49	69 5–	Total
♂	°5	57	23	118	31	31	33	318
♀	3	6	20	126	31	28	21	316
♂ ♀	48	1 4	43	°44	62	59	54	634

Table II
Frequency of pseudoexfoliatio lentis in different populations

Age		40–49		50–59		60–69		70–79		80–99	
Icelanders		No	Ex fol	No	Ex fol	No	Ex fol	No	Ex fol	No.	Ex fol.
N Iceland	♂	51	1	36	2	32	3	28	12	5	[illegible]
	♀	44	2	34	1	27	5	15	6	6	3
Finns*) Central Finland	♂ ♀	–		–		50	5	108	23	[illegible]	[illegible]
Skolt Lapps**) N Finland	♂	22	0	22	2	15	3	4	1	2	1
	♀	20	0	25	3	19	4	9	3	2	1
Cheremisses**) USSR	♂	87	0	44	0	1	1	2	0	–	
Eskimos**)	♂	22	0	20	0	22	0	9	0	1	0
	♀	31	0	26	0	14	0	4	0	–	
S Norway (Aasved 1971)	♂ ♀	3091	0	2827	10	1829	18	476	23	314	24

*) Krause et al (1973)
**) Forsius & Luukka (1973)

pseudoexfoliation in all age groups It is of special interest to note that in Iceland two cases with pseudoexfoliation of the lens capsule were observed in persons of less than 40 years of age We have never seen pseudoexfoliation before in that age group Unless the pupils are dilated you miss 10 20% of the cases with pseudoexfoliation We dilated the pupils regularly only in persons of 45 years or older As most of them are slight cases with changes only in the periphery of the lens capsule and only seen when the pupil is enlarged it seems probable that the figures from Icelanders in the age groups 30 39 and 40 49 should be even higher

Depth of anterior chamber

The corneal thickness and depth of the anterior chamber was measured with Goldmanns pachymeter connected to Haag Streit biomicroscope

Table III
Anterior chamber depth right eye in mm,
Icelanders Husavik 1972

Age	♀			♂		
	No	Mean	SD	No	Mean	SD
0 – 9.49	23	2.94	0.29	5	2.98	0.25
9.50–14.49	67	3.11	0.26	55	3.16	0.26
14.50–19.49	18	3.11	0.26	23	3.22	0.22
19.50–50.49	123	2.86	0.33	115	2.91	0.32
50.50–59.49	29	2.55	0.23	31	2.68	0.31
59.00–69.49	26	2.56	0.46	29	2.65	0.30
69.50–	18	2.41	0.36	23	2.54	0.32
Total	304			301		
Old peoples home Oulu Finland.						
60–69	12	2.56	0.24	30	2.64	0.29
70–	93	2.31	0.31	53	2.45	0.32
Total	105			83		

The depth of the anterior chamber in different age groups in Husavik is similar to European values (Table III)

Eye pressure

The eye pressure for the whole population in Husavik all cases included is shown in Table IV Eighteen persons had earlier verified glaucoma and one new glaucoma simplex case was found Of these persons the youngest was 46 years old three were between 50 and 59 ten between 60 and 69 and five in the next decade Of these 19 seven had glaucoma capsulare three persons were operated for glaucoma and had normalized pressure and 13 were treated with remedies In the total population in only one eye was the eye pressure above 24.4 mmHg a case of glaucoma capsulare seen at our first examination It seems probable that all persons with glaucoma in Husavik came for investigation Even then the frequency 19 glaucomas among 2 000 inhabitants is high

Table IV
Husavik/Iceland 1972
Eye pressure in right eye Schiotz standard

♀ + ♂ Age	No	Mean	SD
0 – 9 49	2	–	–
9 5–14 49	–	–	–
14 5–19 49	1	–	–
19 5–50 49	75	15 64	3 05
50 5–59 49	59	15 34	2 81
59 5–69 49	59	16 17	6 46
69 5–	51	16 14	3 55
Total	247		

and is of the same magnitude as in earlier reports, Skulason (1933) and Sveinsson (1959) All cases were open angle glaucoma The chamber angle was not narrow in any of the eyes In addition seven persons had an intraocular pressure between 21 9 and 24 4 mmHg and were asked to remain under further control as glaucoma was suspected

Seven persons had mature cataract or were aphakic four of them in combination with pseudoexfoliation Three of these had glaucoma capsulare

Discussion

Already in 1917 when Lindberg described pseudoexfoliation he found an accumulation of glaucoma in his material Further studies which have increased especially in the last decade have shown that the combination of pseudo exfoliation and glaucoma is a problem not only for the white race but also for Negroes (Bartholomew 1973) and Indians (Faulkner 1971)

Studies made by our group in Finland (Krause et al 1973) on 220 inmates of old people s homes show that in most cases pseudoexfoliation is not combined with glaucoma in spite of the fact that the mean for the pressure in pseudo exfoliation eyes is somewhat higher than for normal persons The frequency of glaucoma capsulare is percentally increasing in older age groups and in the University Eye Hospital in Oulu 61 5 % of all glaucoma cases > 70 years

are combined with pseudoexfoliation (Krause *1973*) Many *authors* e g Aasved (1971) have shown that glaucoma capsulare also is *more* deleterious than glau coma simplex

In seven of 19 *glaucoma* cases we discovered pseudoexfoliation of the lens capsule Six of them were > 50 years of age This is more than was to be expect ed by change as the frequency of persons with pseudoexfoliation was 18.58 % in persons of 50 years or older

As the overwhelming majority of the exfoliation patients had normal intra ocular pressure and the majority of the glaucomas did not have pseudoexfolia tion we can conclude that pseudoexfoliation may be an important additional factor in the open angle glaucoma problem in Iceland as well as in Finland and Norway but not the deciding point

Angle closure glaucoma is rarer in Iceland than in Finland The narrowness of the chamber angle is according to many authors closely related to the depth of *the anterior chamber It was therefore to be expected that the depth of the* anterior chamber at least in the glaucoma age should be higher in Iceland than it is e g in Eskimos whose chamber depth is very low and closure angle glaucoma extremely common (Alsbirk et al 1973)

It was found that the depth of the anterior chamber in the material from Husavik in Iceland was of the same size as in other European normal materials among others in Finland (*Table III*) The material is small and lacks statistical significance To make clear whether the low *frequency of narrow angle* glau coma in Iceland is caused by a deeper chamber in the oldest age groups a greater number of old people are being studied in Finland and Iceland

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FLAT TYPE ELECTRO OCULOGRAM (EOG)

BY

A. PINCKERS and J M THIJSSEN

The EOG is influenced by the corneo retinal potential of the contralateral eye This contralateral influence results in polarity reversal in severely destructed eyes which do not have their own corneo retinal potential In eyes with a decreased corneo retinal potential the contralateral effect can cause polarity reversal only during the light adapted period Contralateral influence on eyes with abolished light sensitive component but a relatively well preserved light insensitive component results in a paradoxical curve Deterioration of the light sensitive part of the EOG concerns primarily the positive component In severely impaired eyes the EOG value should be corrected to allow for the influence of the contralateral sound eye a formula devised for calculating the corrected values is given in a second paper

Key words electro oculogram – paradoxical EOG – reversed polarity EOG

A markedly abnormal electro oculogram (EOG) is usually called a flat type EOG However when the values recorded are plotted in a graph such EOGs are not always completely flat Special types have been described in the literature as paradoxical lightrise (François Verriest & De Rouck 1957) reversed polarity type (Imaizumi 1964 Imaizumi et al 1966) or inverse light

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modification" (François et al 1966) In this paper a review is given of the flat type EOGs recorded in our *Clinic* and further, an attempt is made to elucidate abnormal types A supplementary *experiment* has been carried out on normal volunteers

Material and Method

The EOG recording technique has been previously described (Thijssen Pinckers & Otto 1974) with this technique the total excursion of the eye movements *is limited to* 40° In this paper the light peak was defined as the mean of the 4 *maximum values* during light adaptation However in cases of flat type EOG *there is no* light peak we therefore define the light peak in such cases as the *mean of the* registrations between the 7th and 11th min after the adaptation lighting of 2 500 lux in the Goldmann Weekers adaptometer sphere has been adjusted (Pinckers & Thijssen 1969)

The *material for the* present study consisted of patients EOGs in which

a) the calculated ratio Lp/Dt is less than 1 00 without occurrence of reversed polarity (flat type EOG)

b) a reversed polarity occurs (reversed polarity EOG)

Results

The EOG graphs were classified by two investigators working independently the result being that both subdivided the flat type EOG into four sub types with an accessory group in which there was reversed polarity (see Table I) The *four sub types* of a flat type EOG were characterized as follows

Type I a completely flat EOG in which the Initial value (Iv) equals the Dark trough (Dt) and the Light peak (Lp) owing to slight differences in the mean Dt and Lp (1 mm = 100 μVolt) the ratio Lp/Dt has a value of less than 1 00 a graph shows this in Fig 1

Type II here the potential measured decreases during the procedure so that the Iv exceeds or equals the Dt while the Dt is higher than the Lp an example of this type is shown in Fig 2

Type III the Lp is lower than the Dt, but there is a kind of Lp to be distinguished in spite of the fact that the ratio Lp/Dt is found to be less than 1 00 see Figs 3 and 8

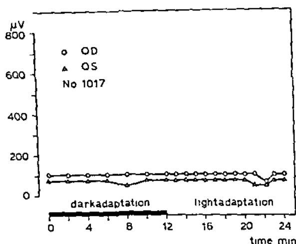


Fig 1
Flat EOG type I (Iv – Dt = Lp)

Type IV an EOG type with a paradoxical curve without polarity reversal the Dt is higher than the Iv while the Lp is lower than the Dt EOGs of this type (see Fig 4) might be called reversed EOGs but this causes confusion with the concept of reversed polarity so that we prefer the term paradoxical EOG

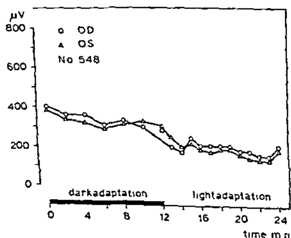


Fig
Flat EOG type II (Iv ≥ Dt > Lp)

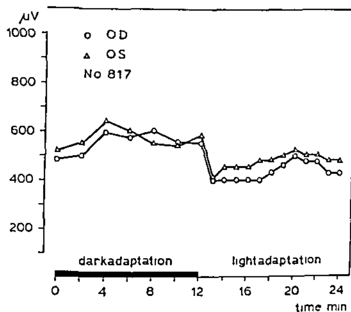


Fig 3
Flat EOG type III (Lp < Dt Lp absent?)

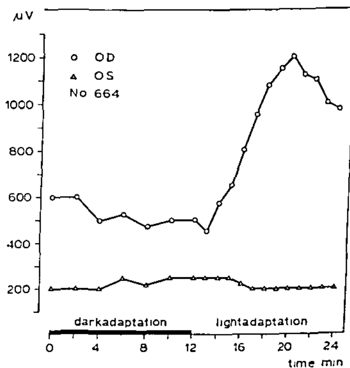
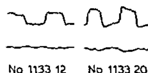


Fig 4
Flat EOG type IV (paradoxical curve)

Finally there is the group of EOGs with polarity reversal A reversed polarity has been encountered only in unilateral lesions In a few cases of unilateral lesions no corneo-retinal standing potential appeared to be present accurate observation revealed that there was no measurable difference in potential during the Dt but that reversal (admittedly slight) occurred during the light adapted phase (Figs 5 and 7)



Fig

Flat EOG reversed polarity type abolished corneo retinal standing potential OS during dark adaptation (12th min) reversed polarity OS during light adaptation (20th min)

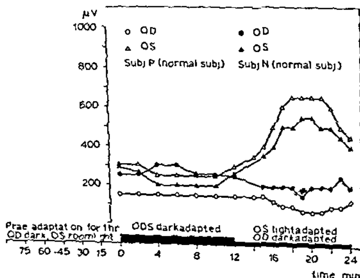


Fig 6

Effect of contralateral eye on dark adapted eye in 2 normal subjects see also text.

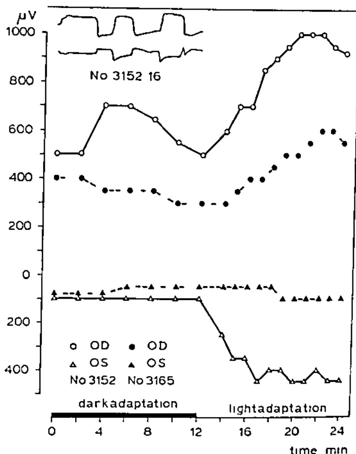


Fig 7

Reversed polarity type EOG in two cases after unilateral enucleation Insert polarity reversal at the 16th min light adapted phase

In Table I the diagnoses made in cases with a flat type EOG are listed The following experiment as described by Thijssen & Pinckers (1974) was carried out as a supplementary study in a number of normal test subjects One eye was completely occluded for 1 hour prior to the start of the experiment the contralateral eye was not occluded Following this pre adaptation period of 1 hour (room light) a normal EOG procedure was carried out during which however the occluded eye was kept occluded throughout. As can be seen in Fig 6 the occluded eye shows a paradoxical curve especially during the light adapted period there is no polarity reversal

Table I

Subtypes of flat EOG ($Lp/Dt < 1.00$)

Disease	Number of patients				Reversed polarity
	Type I (Iv-Dt-Lp)	Type II (Iv≧Dt>Lp)	*Type III* (Lp<Dt absent Lp)	Type IV (paradoxical) curve	
Tapetoretinal degeneration	15	9	2	1	1
Choroidal atrophy	1	1	9	–	–
Stargardt s disease	1	2	1	–	–
Uveitis	1	–	–	–	2
Siderosis/ chalcosis	1	–		–	1
Angiomatosis retinae	1	–	–	–	–
Retrolental fibroplasia	–	1	–	–	–
Retinopathia diabetica	–	1	1	–	–
Perforatio bulbi	–	1	–	–	–
Retinal detachment	–	–	–	1	3
Atrophia bulbi	–	–	–	–	2
Anophthalmus (enucleated)	–	–	–	–	2

Discussion

Since during an EOC measurement, the alterations of the potentials of tw electric dipoles localized on either side of the root of the nose are measured i is important to know whether or not the corresponding electric fields of thes

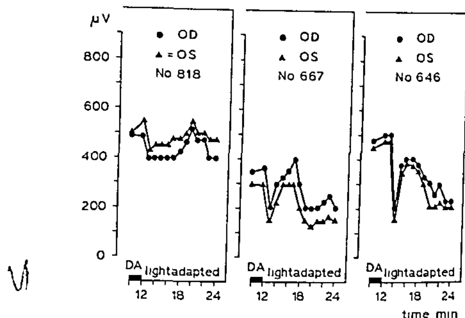


Fig 5
Three cases with remarkable light sensitive negative EOG component classified as flat EOG type III

dipoles influence one another Various authors have arrived at different conclusions in this respect François & De Rouck (1955) found that the electric field of one eye extends to the outer rim of the contralateral orbit Nevertheless they postulate complete independence of the two eyes "the transocular deflections are selective for the corresponding eye since following unilateral enucleation the transocular lead gives no deflection whereas the transocular heterolateral lead gives a normal amplitude (François et al 1957) Arden & Kelsey (1962a) confirm the possibility of recording independently from the two eyes they note that this possibility has been denied by Miles who recorded a small potential around orbits from enucleated eyes Bicas (1972) noted that any periorbital placed electrode records potentials from both ipsilateral and contralateral eyes It was Imaizumi (1964) who introduced the term "reversed polarity type and suggested that the affected eye having no resting potential of its own may have picked up the resting potential of the sound eye on the other side resulting in the appearance of polarity reversal Later on Kelsey (1967) demonstrated a picture of polarity reversal probably due to spread from the sound eye

In the author s material (type reversed polarity see Table I) EOG examina

tions were carried out in two patients who had undergone enucleation of one eye (Fig 7) the results indicate an unmistakable influence exerted by the sound contralateral eye Our reversed polarity group includes nine other patients with severe monocular impaired retinal function the polarity reversal in these cases can always be discerned during the light adapted phase but only rarely during the dark adapted period (Fig 5) Since none of these impaired eyes exhibited normal polarity during the dark adapted period it was impossible to ascertain whether in an eye with its own corneo retinal standing potential polarity rever sal occurs in the light adapted period However Imaizumi (1964) has observed this phenomenon in some cases of pigmentary degeneration

Accordingly it may be regarded as established that

1 the EOG is influenced by the corneo retinal standing potential of the con tralateral eye

2 this contralateral influence results in polarity reversal in severely damaged eyes without own corneo retinal standing potential

3 in eyes with a decreased corneo retinal standing potential the contralateral effect can cause polarity reversal during the light adapted period

The question was considered as to whether a contralateral influence may be demonstrable in normal test subjects After one hour s total occlusion the re sting potential may be expected to have reached a stable level If then a normal EOG procedure is applied to the non occluded eye while the occluded eye is kept occluded throughout all distinct effects can be attributed to the spread of the corneo retinal potential from the contralateral eye Fig 6 presents a para doxical curve of the occluded eye this can only be explained as an effect of the non occluded eye since the basic level of the resting potential in the occluded eye is sufficiently high the influence of the contralateral eye does not result in polarity reversal The graph shown in Fig 6 is virtually identical with that found for the group flat EOG type IV (paradoxical curve see Fig 4) the two patients in this group had monocular abnormalities

François et al (1957 1966) describe a paradoxical light rise during the dark adapted period or a paradoxical fall during the light adapted period in cases of microophthalmus atrophia bulbi atrophia retinae pigmentosa, retinal detach ment, embolism of the central retinal artery and pseudoglioma Arden & Kelsey (19ú b) describe the same picture in unilateral retinal detachment, but offer no explanation of this phenomenon

Conclusion Exertion of contralateral influence in eyes with abolished light rise which still have a sufficiently high light insensitive component results in a paradoxical curve

The flat type EOG types I II and III are encountered in monolateral as well as in bilateral eye affections For this reason one cannot maintain that contra lateral exertion of influence plays an important part in this respect

The findings in flat type EOG type I are not problematical The values of the Iv Dt and Lp are at the same level which implies that the light sensitive com ponent is abolished whereas the light insensitive component is still present although as a rule decreased Owing to inaccuracies (ocular movements measur ing errors), the ratio Lp/Dt is less than 1 00, but this is not of essential signific ance (see Fig 1) It is a common element of the flat type EOG types II and III (see Figs 2 and 3) that immediately after the adjustment of the adaptation lighting to 2 500 lux a fairly pronounced fall occurs The light sensitive poten tial is sensitive to alterations of retinal adaptation and consists of at least two components a slow positive component with a culmination time of approx 9 min and a more rapid negative component with a culmination time of 1 to 2 min (Kolder & North 1967) The negative component persists during light adaptation and can be found in normal subjects (Kolder 1967 Arden & Kelsey 1962a b François et al 1957) but manifests itself clearly when the positive component is absent (François & De Rouck 1973) The fall in potential which the authors observed is in agreement with data in the literature The difference between the flat type EOG types II and III is that in type II the fall is not followed by a rise whereas a rise does occur in type III (Fig 3) As the culmi nation time of this rise is sometimes very short one cannot definitely state that this phenomenon is a genuine light peak

The conclusion is that the negative light-sensitive component is sometimes present when the positive light sensitive component is abolished this results in an Lp/Dt ratio of less than 1 00

Velissaropoulus Palimeris & Andreanos (1971) studied the EOG in a case of unilateral posterior choroiditis The EOG of the sound eye during the phase of recovery hardly differed from that of the acute phase from this the authors concluded that the affected eye did not influence the potential of the sound eye However the intra individual variations of the EOG are considerable (Kelsey 1967 van Lith & Balik 1970 1971)

The observation made by Velissaropoulos et al is undecisive in view of these considerable individual differences The inter individual differences are even more pronounced they depend not only on the individual corneo retinal potent ial but also on the degree of contralateral influence which in its turn is deter mined among other thing by the distance between the two orbits It may be imagined that the contralateral influence for an identical corneo retinal potent ial may be inversely proportional to the degree of hypertelorism

During a normal EOG procedure the potential measured will be lower than the actual corneo-retinal standing potential of the eye in question this has no practical consequences since standardization of an EOG procedure is in normal test subjects with two normal eyes However in one eyed patients or patients with one severely impaired eye the actual corneo-retinal standing potential is measured in the sound contralateral eye and it is found to be higher than in normal circumstances (two normal eyes)

Moreover in severely impaired eyes the EOG value should be corrected for the influence of the contralateral sound eye

In a following paper Thijssen & Pinckers (1974) will calculate the order of magnitude of this correction

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The flat type EOG types I II and III are encountered in monolateral as well as in bilateral eye affections For this reason one cannot maintain that contra lateral exertion of influence plays an important part in this respect

The findings in flat type EOG type I are not problematical The values of the Iv Dt and Lp are at the same level which implies that the light sensitive component is abolished whereas the light insensitive component is still present although as a rule decreased Owing to inaccuracies (ocular movements measuring errors) the ratio Lp/Dt is less than 1 00 but this is not of essential significance (see Fig 1) It is a common element of the flat type EOG types II and III (see Figs 2 and 3) that immediately after the adjustment of the adaptation lighting to 2,500 lux a fairly pronounced fall occurs The light sensitive potential is sensitive to alterations of retinal adaptation and consists of at least two components a slow positive component with a culmination time of approx 9 min and a more rapid negative component with a culmination time of 1 to 2 min (Kolder & North 1967) The negative component persists during light adaptation and can be found in normal subjects (Kolder 1967 Arden & Kelsey 1962a b François et al 1957) but manifests itself clearly when the positive component is absent (François & De Rouck 1973) The fall in potential which the authors observed is in agreement with data in the literature. The difference between the flat type EOG types II and III is that in type II the fall is not followed by a rise, whereas a rise does occur in type III (Fig 3) As the culmination time of this rise is sometimes very short one cannot definitely state that this phenomenon is a genuine light peak

The conclusion is that the negative light sensitive component is sometimes present when the positive light-sensitive component is abolished this results in an Lp/Dt ratio of less than 1 00

Velissaropoulus Palimeris & Andreanos (1971) studied the EOG in a case of unilateral posterior choroiditis The EOG of the sound eye during the phase of recovery hardly differed from that of the acute phase from this the authors concluded that the affected eye did not influence the potential of the sound eye However the intra individual variations of the EOG are considerable (Kelsey 1967 van Lith & Balik 1970 1971)

The observation made by Velissaropoulos et al is undecisive in view of these considerable individual differences The inter individual differences are even more pronounced they depend not only on the individual corneo retinal potential but also on the degree of contralateral influence which in its turn is determined among other thing by the distance between the two orbits It may be imagined that the contralateral influence for an identical corneo retinal potential may be inversely proportional to the degree of hypertelorism

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CONTRALATERAL EFFECTS IN THE ELECTRO-OCULOGRAM

BY

J. M. THIJSSEN and A. PINCKERS

The derivation of the contralateral effect from EOG data is presented. The mathematical procedure can be adapted to special experimental conditions, such as *unilateral occlusion* and *asymmetric fixation*, or for instance to a group of patients with unilateral enucleation. The contralateral spread of the standing potential of the eye is significantly shown by the experimental results, and is expressed by a factor f. This factor is of the order of 15 % and represents the fraction of the ipsilateral potential that is measured contralaterally. A method is given to correct the EOG data in case of unilateral impairment.

Key words: electro-oculogram – contralateral effect – paradoxical EOG – reversed polarity EOG.

The possibility that the measurement of the electro-oculogram (EOG) of one eye is partly determined by the standing potential of the other eye has been mentioned by various authors. However, the evidence is somewhat confusing, as the contralateral effect has been found by Miles (1939), Kelsey (1968), Arden & Kelsey (1962a) and Bicas (19[illegible]), whereas its occurrence is denied by Arden

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The derivation of the contralateral effect from EOG data is presented The mathematical procedure can be adapted to special experimental conditions such as unilateral occlusion and asymmetric fixation or for instance to a group of patients with unilateral enucleation The contralateral spread of the standing potential of the eye is significantly shown by the experimental results and is expressed by a factor f This factor is of the order of 15 % and represents the fraction of the ipsilateral potential that is measured contralaterally A method is given to correct the EOG data in case of unilateral impairment.

Key words electro oculogram – contralateral effect – paradoxical EOG – reversed polarity EOG

The possibility that the measurement of the electro oculogram (EOG) of one eye is partly determined by the standing potential of the other eye has been mentioned by various authors However the evidence is somewhat confusing as the contralateral effect has been found by Miles (1939) Kelsey (1968) Arden & Kelsey (1962a) and Bicas (1972) whereas its occurrence is denied by Arden

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& Kelsey (1962b) François et al (1957) and Miles (1958) It should be mentioned that the finding of Miles (1938) was reversed within a year by the results that he obtained from patients with unilateral enucleation These patients displayed an EOG of reversed polarity at the prosthesis which result was explained by contralateral spread of the electrical field of the healthy eye. Also the results of Arden & Kelsey (1962 a b) are contradictory since they observed a reversed light peak (or light trough) from an eye with a complete retinal detachment. We have interpreted this finding as the contralateral effect of the *light peak of the healthy eye on the invariable standing potential of the other eye*. In a previous paper (Pinckers & Thijssen 1974) we have called this a "flat paradoxical EOG" and we have presented two patients with such EOG curves. Furthermore we found various other types of "flat" EOG responses (light peak/dark trough ratio $< 1\,0$) the completely flat EOG (no light peak at all) the continuously decreasing flat EOG (initial value > dark trough > light peak) discontinuous flat EOG (small light peak of lower magnitude than the dark trough) and the completely reversed flat EOG (polarity reversal c.f Imaizumi 1966) We concluded that these findings can be explained by the contralateral influence of the potential of the healthy eye. This conclusion prompts two questions

1 what is the magnitude of the "real" EOG of the damaged eye and

2 what magnitude would be measured at the healthy eye in the normal situation?

In other words is it possible to calculate a corrected value of the EOG of both eyes in the case of a unilateral disease yielding a very low or zero standing potential? A secondary problem that will be considered in this paper is the observation that the temporal electrode registers a larger potential than the nasal electrode (François et al 1957 and Bicas 1972)

We will start with a mathematical treatment of the contralateral effect under various conditions and the derivation of a method to correct the measured EOG in case of unilateral diseases Thereafter we will present the results of experiments yielding the numerical values which can be used to perform this correction and the limits of accuracy of EOG measurements without making use of the correction will be given

Mathematical treatment of the contralateral effect

1 General Two assumptions are essential for this treatment

1) the real standing potentials of the eyes resulting in the EOG measurement are independent.

2) the contralateral effect results in a decrease in the EOG potential amounting to a fixed fraction of the real standing potentials

The first assumption is very plausible since it is generally assumed that the EOG is generated in the distal layers of the retina (Brown 1968)

The second assumption is based on the first, because in the case of independence the additivity principle of electric potentials yields a subtraction by a particular fraction of the standing potential of the contralateral eye For reason of symmetry we will assume this fraction to be identical for both eyes The subtraction results from the experimental procedure Considering the eyes as dipoles with the apex of the cornea as the positive pole moving the eyes simultaneously means that the negative pole moves towards the positive side of the contralateral eye and vice versa

2 *The normal EOG measurement* The various values of the standing potential used in the following description are defined in Fig 1 The measured quantities are given in capitals (e g DT_R LP_L) whereas the real" values which are to be calculated will be given in lower case (e g dt_L lp_R) The fractional contralateral effect is denoted by f The measurement of the EOG can be represented by the following set of equations

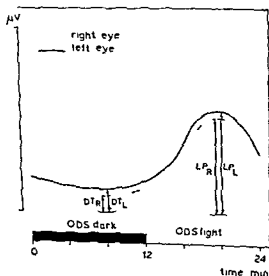


Fig 1

Schemat c EOG curves resulting from a normal measurement Dark trough values (DT) are defined as the average of the values at the 6th 8th 10th and 12th min Light peak values (LP) are the average of the data at the 19th 20th 21th and 22nd min

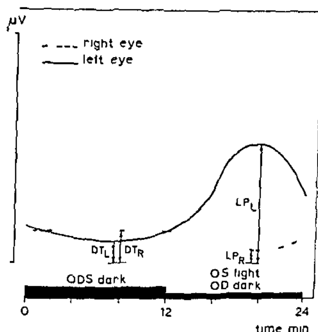


Fig 2

Schematic curves for unilateral occlusion (OD) yielding a paradoxical flat EOG at the right eye Definitions as in Fig 1

Left eye	$DT_L = dt_L - f \quad dt_R$	(1)
	$DT_R = -f \quad dt_L + dt_R$	(2)
and	$LP_L = lp_L - f \quad lp_R$	(3)
	$LP_R = -f \quad lp_L + lp_R$	(4)

It will be clear that without knowledge of the fraction f this set contains five unknown variables and cannot therefore be solved

3 EOG measurement with unilateral occlusion In this case (see Fig 2) if e g the right eye is occluded lp_R is essentially equal to dt_R and the set of equations becomes

	$DT_L = dt_L - f \quad dt_R$	(1a)
	$DT_R = -f \quad dt_L + dt_R$	(2a)
and	$LP_L = lp_L - f \quad dt_R$	(3a)
	$LP_R = -f \quad lp_L + dt_R$	(4a)

Resolving these equations yields

$$f = \frac{DT_R - LP_R}{LP_L - DT_L} \tag{5}$$

$$lp_R = dt_R = \frac{f \; DT_L + DT_R}{1 - f^2} \tag{6}$$

$$dt_L = DT_L + f \; dt_R \tag{7}$$

$$lp_L = LP_L + f \; dt_R \tag{8}$$

4 EOG measurement with asymmetric fixation or in the case of unilateral enucleation With asymmetric fixation one eye (for instance the right eye) is not displaced during the measurement of the EOG Hence any potential registered at that eye is due to the contralateral spread from the left eye In this respect this situation is identical with the case of a unilateral enucleation The resulting EOG is shown schematically in Fig 3 The set of equations now becomes

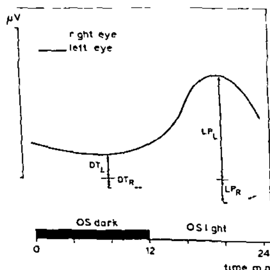


Fig 3
Schematic curves for unilateral enucleation (OD) resulting in a reversed polarity EOG
Definitions as in Fig 1

$$DT_L = dt_L \qquad (1b)$$

$$DT_R = -f\ dt_L \qquad (2b)$$

and

$$LP_L = lp_L \qquad (3b)$$

$$LP_R = -f\ lp_L \qquad (4b)$$

It now follows directly from these equations that

$$f = -\frac{DT_R}{DT_L} \qquad (9)$$

and also

$$f = -\frac{LP_R}{LP_L} \qquad (10)$$

5 Correction of the normal EOG measurement for the contralateral effect The value of the factor f being determined with one of the above mentioned procedures and anticipating the result that the inter subject deviation is small the equations (1) to (4) can be rewritten in order to calculate the real EOG values It follows from equations (1) and (2) that

$$dt_L = \frac{DT_L + f\ DT_R}{1 - f} \qquad (11)$$

$$dt_R = \frac{f\ DT_L + DT_R}{1 - f^2} \qquad (12)$$

and from equations (3) and (4) that

$$lp_L = \frac{LP_L + f\ LP_R}{1 - f} \qquad (13)$$

$$lp_R = \frac{f\ LP_L + LP_R}{1 - f^2} \qquad (14)$$

This set of equations can now be used to calculate the real light peak/dark trough ratio r
Thus

$$r_L = \frac{lp_L}{dt_L} \quad \frac{LP_L + f\ LP_R}{DT_L + f\ DT_R} \qquad (15)$$

$$r_R = \frac{lp_R}{dt_R} = \frac{f\ LP_L + LP_R}{f\ DT_L + DT_R} \qquad (16)$$

From equations (15) and (16) it follows that if either the measured LP and DT values of one eye are not much different from the values of the other eye or the ratios are approximately equal then the real ratio r is approximately

equal to the measured ratio $R = \frac{DT}{LP}$ Otherwise in the case of unilateral diseases the ratio for the affected eye can only be reasonably determined by using the equations (15) and (16)

A somewhat simpler procedure for this correction will be given here

Defining the light peak ratio

$$X = \frac{LP_L}{LP_R} \text{ so } LP_R = \frac{LP_L}{X} \tag{17}$$

and the dark trough ratio

$$Y = \frac{DT_L}{DT_R} \text{ so } DT_R = \frac{DT_L}{Y} \tag{18}$$

We can rewrite equation (16)

$$r_L = \frac{LP_L\,(1 + f/X)}{DT_L\,(1 + f/Y)} = R_L \quad \frac{(1 + f/X)}{(1 + f/Y)} \tag{16a}$$

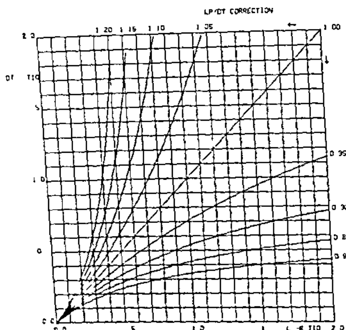


Fig 4

Correction of the light peak dark trough ratio for a fractional contralateral effect f = 15 Horizontal light peak ratio of the eyes Vertical dark trough ratio of the eyes

$$DT_L = dt_L \tag{1b}$$

$$DT_R = - f \; dt_L \tag{2b}$$

and

$$LP_L = lp_L \tag{3b}$$

$$LP_R = - f \; lp_L \tag{4b}$$

It now follows directly from these equations that

$$f = - \frac{DT_R}{DT_L} \tag{9}$$

and also

$$f = - \frac{LP_R}{LP_L} \tag{10}$$

5 Correction of the normal EOG measurement for the contralateral effect The value of the factor f being determined with one of the above mentioned procedures and anticipating the result that the inter subject deviation is small the equations (1) to (4) can be rewritten in order to calculate the real EOG values It follows from equations (1) and (2) that

$$dt_L = \frac{DT_L + f \; DT_R}{1 - f^2} \tag{11}$$

$$dt_R = \frac{f \; DT_L + DT_R}{1 - f^2} \tag{12}$$

and from equations (3) and (4) that

$$lp_L = \frac{LP_L + f \; LP_R}{1 - f} \tag{13}$$

$$lp_R = \frac{f \; LP_L + LP_R}{1 - f^2} \tag{14}$$

This set of equations can now be used to calculate the real light peak/dark trough ratio r

Thus

$$r_L = \frac{lp_L}{dt_L} \quad \frac{LP_L + f \; LP_R}{DT_L + f \; DT_R} \tag{15}$$

$$r_R = \frac{lp_R}{dt_R} = \frac{f \; LP_L + LP_R}{f \; DT_L + DT_R} \tag{16}$$

From equations (15) and (16) it follows that if either the measured LP and DT values of one eye are not much different from the values of the other eye or the ratios are approximately equal then the real ratio r is approximately

Results An example of the EOG curves obtained with this procedure is shown Fig 5 The dark trough is defined as the average of the EOG potential at e 6th to the 12th min of registration the light peak is defined as the average the values at the 19th to 22nd min The data from seven subjects are given Table I As can be seen in this Table the light peak/dark trough *ratio of the ght (occluded) eye is smaller than 1 0 which indicates a light trough instead a light peak* The fractional contralateral effect, expressed by the correction ctor f is calculated by using equation (5) The relatively large spread of the values may be due in part to the non perfect occlusion of the right eye How er a lower limit of 10 % and an upper limit of 20 % for the factor f seems to e reasonable

xperiment 2 Asymmetric fixation

Procedure The subjects were seated with the head on a chin rest and were nstructed to press the forehead against a metal bow As illustrated in Fig 6 the ight eye was positioned exactly in line with two fixation marks F_1 and F laced at 18 cm and 550 cm respectively A small ring R was used during this rocedure as an additional cue to the proper adjustment, and removed there fter Next the subject was asked to fixate alternatively F_1 and F while

Table I

Contralateral effect measured by means of unilateral occlusion
Normal subjects right eye (OD) occluded

OS Dark trough (μV)	OS Light peak (μV)	OD Dark trough (μV)	OD Light peak (μV)	OS *Ratio*	OD Ratio	f (%)
50	6 0	150	5	2 60	0.50	19
00	6 5	250	190	2 60	0 71	14
300	600	240	180	2 00	0 74	20
3 5	850	250	250	2 60	1 00	0
450	890	400	340	1 93	0 88	14
550	10 0	250	250	1 99	0 90	6
300	6 0	240	10	2 67	0 57	9

av 12 %

with R_L being the measured LP/DT ratio of the left eye Equation (16a) is used to calculate the correction lines in Fig 4 As can be seen the LP/DT ratio is most affected by the contralateral effect if either the light peak value or the dark trough value of one eye differs from the value of the other eye Whereas, in the case of almost equal light peak and dark trough ratios the light peak/dark trough ratio is almost independent of the difference between the eyes.

Experiment 1 Unilateral occlusion

1 Procedure One hour before the beginning of the experiment one of the subject s eyes was carefully occluded The electrodes were then fixed to the skin near the canthi of the eyes under dim red light conditions and thereafter the occlusion was replaced on the eye Next a normal EOG measurement was performed starting with 12 min of dark adaptation and followed by 12 min of light adaptation (2500 lux from an adaptometer sphere c f Thijssen et al 1974)

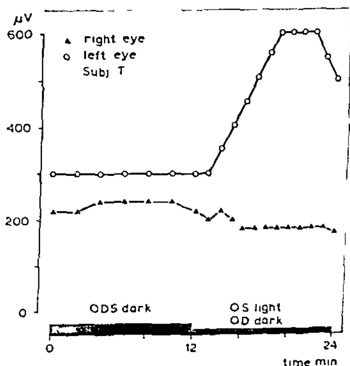


Fig 5
Experimental curves resulting from unilateral occlusion

Table II

Contralateral effect measured by means of asymmetric fixation (normal subjects)

OS (μV)	OD (μV)	f (%)
+ 400	− 50	12.5
+ [illegible]00	− 40	0
+ 400	− 90	2[illegible] 5
+ 3[illegible]0	− 40	12
+ 200	− 20	10
+ 2[illegible]0	− 50	18.5
+ 400	− 60	15
+ 300	− 60	20
+ 3[illegible]0	− 30	1[illegible].5
		av 16 %

small jump However after a short time this eye remains in a fixed position and a small potential difference can be registrated with a polarity that is reversed with respect to the potential measured at the left eye

The data from nine subjects are given in Table 2 and the factor f is derived from equation (9) On an average the f values obtained with this method tend to be higher than the values found with experiment 1

Unilateral enucleation

The results described by Miles (1939) are presented in Table III We have used all the data available in that paper so some figures are in fact a measurement repeated with the same patient The results from six of our own patients are given as well but here both the dark trough and the light peak values are presented The data marked with * are from a patient suffering from a traumatic retinal detachment.

The overall average of the correction factor in Table III is 15 % however it should be noted that the data from our patients tend to be significantly higher than those collected by Miles

pursuing binocular fusion By this means the right eye remained in a fixed position whereas the left eye converged 20° Two pairs of electrodes were placed near the canthi of both eyes

2 *Results* An example of the registration is shown in Fig 7 It can be seen that at the moment of the switch from F_1 to F_2 and vice versa the right eye makes a

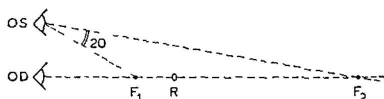


Fig 6

Scheme of the procedure for asymmetric fixation Fixation marks F_1 and F ring R

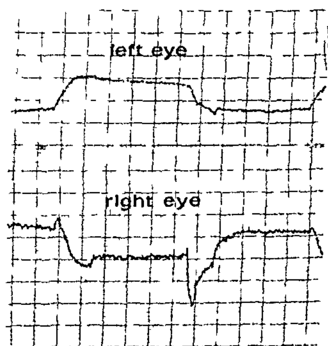


Fig 7

Registration traces of the experiment with asymmetric fixation Upper trace left eye rotation of 20° 270 μV Lower trace right eye (3 times enlarged) 50 μV

If the eyes are considered to be a dipole then it is possible to assess the contralateral effect in a simplified electric field scheme According to electric field theory the potential at a distance r from the centre of the dipole is pro portional to the cosine of the angle between the dipole vector and the distance vector and moreover inversely proportional to the square of the distance

$$\Phi = \frac{\vec{p} \quad \vec{r}}{r^3} \quad \frac{1}{4 \pi \varepsilon \quad \varepsilon} = \frac{p \quad \cos \alpha}{r^2}$$

By simplifying the measuring geometry so that all the electrodes are on the same straight line and the dipole is located in the centre of the eye then the potential at the contralateral nasal electrode will be 16 % of the potential at the ipsilateral nasal electrode The potential at the contralateral temporal electrode would be 2 % and will therefore be ignored Since the eye is not embedded in an homogeneous medium and the nose has a complex geometry the figure of 16 % should be considered as an approximation which appears to be of the same order of effect as determined experimentally [1)]

We have shown by mathematical treatment that the contralateral effect does not influence the light peak/dark trough ratio if the ratio of one eye does not differ much from the ratio of the other eye This means that the ratio has to be corrected for the contralateral effect when either the light peak or the dark trough of one eye differs considerably from the value found for the other eye in other words when the light peak/dark trough ratios are markedly differing

We can now explain why a temporally placed electrode registers a larger potential than one placed nasally A 15 % decrease in the nasal potential (mono polar derivation) with respect to the temporally measured potential will be caused by the contralateral effect. Considerations based on the geometry of the electrode placement (c f Bicas 1972) should also be included but we will con fine ourselves to the contralateral effect.

Acknowledgements

We wish to thank Mr B Nabbe for his valuable technical assistance Dr Dr C Bakker for writing the computer programme, and Mrs M Huijts for carefully preparing the manuscript.

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1) Note added in proof a more elaborate calculation will be published soon (Thijssen & I in kers n press)

Table III

Contralateral effect of healthy eye on enucleated side. Data from Miles (1939) left columns. Data collected by authors right columns.

Healthy eye (μV)	Prosthesis (μV)	f (%)	Healthy eye (μV)	Prosthesis (μV)	f (%)
+870	−65	7.5	+500	−100	20
830	65	7.8	–	–	–
750	50	6.7	280	50	18
670	50	7.5	480	100	21
840	90	11	350	100	29*
790	–	0.0	1050	350	33*
410	55	13	400	50	13
760	105	14	950	110	12
920	140	15	720	150	21
470	120	26	400	100	25
			350	50	14
			650	100	15
		av. 11 %			av. 20 %*

Discussion

The contralateral effect is clearly demonstrated both by the experimental results and by the data from patients with unilateral enucleation. The magnitude of the effect is expressed by the factor f. The values found for this factor are in the range of 10 % to 30 %. The results of the experiment with unilateral occlusion may be somewhat affected by inadequate occlusion. This may be illustrated by the fact that we had to exclude two subjects from Table I because they displayed a normal light peak at the occluded side instead of a light trough. The data of Miles yield lower f values than our data (Table III), the reason for this is not obvious. We propose therefore, as a rule of thumb, a contralateral effect of 15 %.

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BENIGN MUCOUS MEMBRANE PEMPHIGOID

III Biopsy

BY

S RY ANDERSEN O A JENSEN E BJORN KRISTENSEN & M S NORN

Conjunctival biopsy specimens were taken from the inferior fornix of 16 eyes affected with benign mucous membrane pemphigoid (BMMP) Biopsy revealed in all cases metaplasia changing the normal columnar epithelium into squamous epithelium, with parakeratinization in eight specimens and keratinization as well in four Correla ion was noticed between keratiniz ation and reduced secretions of both tears and mucus.
Mucus producing goblet cells were remarkably scarce in eight cases and totally absent in the remaining eight. This corresponds approximately to the result of an estimation of the mucus secretion by measuring the mu cous thread after vital staining with a tetrazolium alcian blue mixture. Four of the 16 biopsies revealed fibrosis of the submucous connective tis sue *The connective tissue was found to contain relatively few neutrophilic granulocytes* In two cases eosinophil c granulocytes were present in small numbers while several lymphocytes and plasma cells were seen, in five even an adeno d layer *Neither bullae nor acantholysis were demonstrated* Such cytological methods as examinations of smears and quantitative pi pette samples can disclose epithelial changes, and examination of the mucous thread can be used as a goblet cell function test. *Hence these* methods can, to a certain extent replace the procedure for estimating the

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specimens therefore had to be taken from each region one specimen being rarely divisible for the three purposes

The eye was anaesthetized by instilling 0 4 % Novesin® (oxibuprocaini chloridum) prior to taking biopsy specimens Injection of an anaesthetic was forborne to avoid artificial oedema of the connective tissue

The biopsy specimen for light microscopy was immediately fixed in 4 % buffered neutral formaldehyde to which 7 5 % sucrose had been added The ordinary paraffin technique was employed Some of the histological sections were stained by haematoxylin eosin and some by haematoxylin phloxine saphranine in some cases supplemented by other staining methods (PAS Unna Pappenheim acid iron orcein reticulum stain)

A considerable loss of tissue was seen immediately after the biopsy specimen had been taken the remaining part of the conjunctiva being tense and receding from the large exposed epithelial defect Only slight bleeding occurred which was easily arrested by compression Suturing was not employed The patient was treated with chloramphenicol ointment

On control one week later the defect was seen to have been covered by epithelium The epithelial layer was often thin over the area from which the biopsy specimen had been taken but there were no complications or inconvenience worth mentioning

Result

In normal eyes the conjunctival epithelium is columnar in the inferior conjunctival fornix (Norn 1960) The biopsy specimens from the 16 pemphigoid affected eyes were all covered by a squamous epithelium which was parakeratinized in eight cases and keratinized as well in four

In four cases the subjacent connective tissue was definitely more fibrous than in normal eyes

The conjunctival epithelium had in other words been converted more or less into a dermatoid layer covering a connective tissue cicatrix

No proper bullae were detected either in the epithelium or between this and the basal membrane Bulla like artefacts were seen in two cases

Epithelium

Biopsy showed all 16 specimens to be covered by squamous epithelium This was often atrophic consisting of only a few cell layers (Fig 1)

In agreement with this biopsy finding squamous cells were found in smears from all 16 conjunctivae and abnormal epithelial elements *in conjunctival*

goblet cell content in a conjunctival biopsy specimen Taking biopsy specimens has the disadvantages of causing pain tissue damage and additional cicatricial shrinkage Furthermore a small biopsy specimen is not always representative

Key words conjunctiva – ocular pemphigoid – benign mucous membrane pemphigoid essential conjunctival shrinkage – biopsy

The epithelium of eyes with benign mucous membrane pemphigoid (BMMP) has previously been studied by estimating the cell contents of the conjunctival fluid and examining conjunctival smear (Norn & Kristensen 1974) In the present investigation, the epithelium and the subjacent connective tissue were examined by light microscopy of conjunctival biopsy specimens

Material

The material examined comprised conjunctival biopsy specimens from 16 eyes affected with BMMP It is identical with that previously studied (Kristensen & Norn 1974)

Clinically all the patients showed a marked conjunctival shrinkage which was not due to any known eye lesion The cases were chronic many having persisted for years None were at the acute stage at the time of investigation

Method

Conjunctival biopsy specimens were taken from a symblepharon band or from the marginal zone of such a band in the inferior fornix of the patient s very shrunken conjunctiva

The specimen was taken with a pair of pointed curved scissors and with tweezers We tried to fix the tissue by means of a curved needle (twisted double hook) but often had to give up because the shrunken conjunctiva was so thoroughly attached to the underlying layer that a firmer grip with tweezers was required

The biopsy specimens were subjected to both conventional light microscopy and to electron microscopy and immune fluorescence microscopy Two or three

specimens therefore had to be taken from each region one specimen being rarely divisible for the three purposes

The eye was anaesthetized by instilling 0 4 % Novesin® (*oxibuprocaim chloridum*) prior to taking biopsy specimens Injection of an anaesthetic was forborne to avoid artificial oedema of the connective tissue

The biopsy specimen for light microscopy was immediately fixed in 4 % buffered, neutral *formaldehyde to which 7 5 % sucrose* had been added The ordinary paraffin technique was employed Some of the histological sections *were stained by* haematoxylin eosin and some by haematoxylin phloxine saph ranine in some cases supplemented by other staining methods (PAS Unna Pappenheim acid iron orcein reticulum stain)

A considerable loss of tissue was seen immediately after the biopsy specimen had been taken the remaining part of the conjunctiva being tense and receding from the large exposed epithelial defect. Only slight bleeding occurred which was easily arrested by compression Suturing was not employed The patient was treated with chloramphenicol ointment

On control one week later the defect was seen to have been covered by epith elium The epithelial *layer was often thin over the area from which the biopsy* specimen had been taken but there were no complications or inconvenience *worth mentioning*

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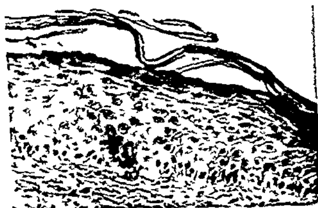


Fig 2

Biopsy from the inferior conjunctival fornix from a patient suffering from benign mucous membrane pemphigoid *Keratinization of the epithelium* Superficially layers of flat keratinized cells without nuclei Below these parakeratinized cell layers with pyknotic nuclei Profound layers of normal appearance. (Haematoxylin eosin 300 X
E P I Lab no 592/ ?)

Keratinization Biopsy disclosed superficially in the specimen a few layers of flat keratinized cells without nuclei Below these a parakeratinized cell layer with pyknotic nuclei was found At a still lower level we observed conjunctival epithelial cells of *normal appearance* (*Fig* 2)

Four of the 16 biopsy specimens showed these highly abnormal conditions In three of these cases leucoplakia was present in the patient s conjunctiva

In three cases cytologic examination showed the epithelium to be in a definitely pathological state

Occurrence of keratinization was not limited to cases in a clinically advanced abnormal state but was also seen in one with a normal cornea and merely initial conjunctival changes

A correlation seemed to exist between biopsy findings and secretions of both tears and mucus (The tear secretion was reduced in all four eyes with keratinization and the mucous secretion in three of these Of the four eyes in which biopsy showed neither keratinization nor parakeratinization two had a normal tear secretion and all four a normal mucous secretion.)

Goblet cells

The number of mucus producing cells in the epithelium was remarkably low In eight cases no goblet cells whatever were detectable In the remaining eight



Fig 1

Biopsy from the inferior conjunctival fornix from a patient suffering from benign mucous membrane pemphigoid Few layers of squamous epithelium covered by a mucous layer In the connective tissue many scattered lymphocytes (Haematoxylin eosin 300 X E P I Lab no 527/72)

fluid using the quantitative pipette method in 14 of the 16 eyes In the remaining two cases the conversion into squamous epithelium must have been less comprehensive and consequently not sufficiently manifest in the conjunctival fluid

Parakeratinization was observed in 12 of the 16 cases The parakeratinization was confirmed cytologically in nine of the 12 cases while this abnormality was demonstrated cytologically in two of the four cases in which biopsy had given a negative result

Thus the results of biopsy and cytology were not completely identical The two methods supplement each other

No correlation was noticed between the leucoplakia diagnosed clinically in the slit lamp and the parakeratinization revealed by biopsy



Fig 2

Biopsy from the inferior conjunctival fornix from a patient suffering from benign mucous membrane pemphigoid Keratinization of the epithelium Superficially layers of flat keratinized cells without nuclei Below these parakeratinized cell layers with pykn tic nuclei Profound layers of normal appearance (Haematoxylin eosin 300 X E.P I Lab no 592/7?)

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Table I
Goblet cells and mucus production Comparison of goblet cell contents estimated by biopsy and mucus production (microscopical measurement of vital stained mucous thread) from 16 BMMP affected eyes

	No goblet cells	+ Goblet cells
Biopsy		
Mucous thread diminished	7	2
Mucous thread normal	1	6

only few scattered goblet cells were seen much fewer than normally expected in a conjunctival specimen from the inferior fornix

The mucous secretion was estimated by measuring in the microscope the area of the mucous thread after vital staining with a tetrazolium alcian blue mixture (Kristensen & Norn 1974)

A fair agreement was noticed between on one hand the number of goblet cells found and on the other the size of the vital stained mucous thread from the inferior conjunctival fornix (Table I)

Connective tissue

Marked fibrosis was diagnosed in four of the 16 cases In the other cases the question of cicatrization could not be settled by microscopy However on taking biopsy specimens it was our impression that the conjunctiva was firmly attached to the substratum

The *basal membrane* was rudimentary or absent in eight cases and particularly thick in one

Odd *eosinophils* were found scattered in two biopsy specimens In one of these cases eosinophils were also present in the cytological preparation The finding suggests an allergic reaction

Neutrophils were often found scattered in the connective tissue in fairly small numbers The quantitative cytological preparation is better suited for deciding whether the number is pathologically increased (Norn 1960)

Lymphocytes together with plasma cells and connective tissue cells were found scattered in all the specimens In five specimens the number of lymphocytes was increased nests of these constituting a dense border in the connective tissue Such an "adenoid layer may occur as a normal phenomenon in the

conjunctiva The observation of many lymphocytes in the connective tissue was not correlated to any clinical or cytological findings Lymphocytes were not seen in increased numbers in quantitative pipette samples of conjunctival fluid

Discussion

Biopsy revealed that most BMMP affected eyes have a parakeratinized or even keratinized conjunctival epithelium Cytologic examinations gave results con firming and supplementing those of biopsy Thus the over all result was that they all have an abnormal epithelium.

In BMMP the normal columnar epithelium of the fornix and the tarsus be comes converted into squamous epithelium which shows parakeratinization or even keratinization This conversion is diffuse not limited to the areas covering symblepharon bands or leucoplakia patches

Estimation of the number of goblet cells in a small conjunctival biopsy spe cimen is subject to some uncertainty these cells being very irregularly distribut ed in the conjunctiva We may find regions rich in goblet cells bordering on regions showing extremely few of these cells

Only a very large specimen (or one comprising the whole conjunctiva) allows an exact estimation (cf Kessing 1968)

Nevertheless there is fair agreement between the estimated number of mu cus producing goblet cells in the biopsy specimens under review and the size of the mucous thread in the inferior fornix as assessed in the slit lamp after vital staining or by additional microscopical measurement following transfer of the mucous thread to a slide

The mucus production may however vary somewhat from day to day as illustrated by variations in the size of the mucous thread In some cases we found a normal mucus production on one examination against practically none on examination of the same pemphigoid eye one week later The reported results represent an average of two or more examinations of the individual eye

We cannot decide on the basis of the observations made in the present in vestigation whether the decreasing mucus secretion or the decreasing tear secre tion or possibly other factors are responsible for the grave complications such as shrinkage of the conjunctival connective tissue the dermatoid growth extend ing over part of the cornea keratinization etc

The material under review comprises only specimens from long standing cases. Bi psy at the acute stage with bulla formation might probably disclose interesting factors

Biopsy of specimens from the present material revealed keratinization para keratinization and a reduced number of mucus producing goblet cells Conver sion of epithelium can however also be disclosed by cytologic examination (smear or quantitative pipette sample) A reduced mucous secretion can be shown by estimating the size of the mucous thread in the inferior conjunctival fornix after vital staining with a mixture of tetrazolium and alcian blue

Taking a conjunctival biopsy specimen can hardly be regarded as a trivial intervention in eyes affected with BMMP The conjunctival shrinkage leaves a large wound uncovered by epithelium and biopsy will presumably cause further shrinkage The small specimen is hardly representative We are there fore fortunate in having other examination methods at our disposal

Comparison of the results of biopsy cytology, and vital staining

The present investigation bore out the view that vital staining with tetrazolium alcian blue is reliable procedure for estimating the topical mucous secretion assessed by blue specifically stained mucus in the mucous thread in the inferior conjunctival fornix *This method is therefore useful as a goblet cell function test* either by direct observation in the slit lamp or by measuring the area on a slide examined in the microscope

Use of this vital stain mixture is however contra indicated where connective tissue is completely uncovered by epithelium because when in direct contact the connective tissue may remain discoloured for several months (Norn 1973) Such tatooing has not been noticed in the present BMMP material

Table II

Comparison of the results of biopsy cytology and vital staining in cases of pemphigoid affected eyes (Estimation of mucus epithelium and emigrating cells)

	Biopsy	Cytology	Vital staining
Can be repeated	hardly	yes	yes
Reliability	not representative of goblet cells lymphocytes etc	quantitative is reliable for emigrating cells	reliable goblet cell function test
Side effects	pain	none	tattooing of connective tissue uncovered by epithelium

The cytologic technique can disclose parakeratinization The methods are harmless It is however advisable to take two or more specimens from each eye to avoid false negative results

A quantitative method offers the additional advantage of permitting us to assess the significance of cells which have emigrated into the conjunctival fluid

Table II illustrates a comparison of the results of biopsy cytology and vital staining The three methods of examination supplement each other

Our previously described clinical examinations in connection with biopsy cytology and vital staining give the impression of a richly facetted pathological conversion of the conjunctiva and the cornea in the disease termed essential conjunctival shrinkage or benign mucous membrane pemphigoid (BMMP)

In a future paper other members of the Copenhagen pemphigoid group will report their results of electron microscopical and immune microscopical examinations of the same conjunctivae

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TREATMENT OF OCULAR HYPERTENSION WITH ORAL BETA-ADRENERGIC BLOCKING AGENTS

BY

MAURIZIO PANDOLFI and ARNE ÖHRSTRÖM

Propranolol, a β adrenergic blocking agent, was given by mouth to 2[illegible] patients with elevated IOP resistent to conventional medical treatment or where conventional treatment was contraindicated. A lasting reduction of IOP was obtained in 11 cases; open angle glaucoma or elevated IOP responded best. No significant side effects were observed. Having in mind the contraindications of β adrenergic blocking agents, propranolol can be usefully employed in ophthalmology for controlling elevated IOP.

Key words: glaucoma simplex – beta adrenergic blockers

Several reports are available on the favourable effect of topical propranolol, a beta adrenergic blocking agent (Bietti 1972), on increased intraocular pressure (IOP). Also the general administration of this agent in doses of 10–30 mg daily has been claimed to have the same effect (Phillips et al. 1967, Cote 1968). But the latter studies have not resulted in any appreciable clinical use of the drug by general administration because the tension reducing effect was often insufficient or transient. Larger doses of oral propranolol have recently been reported by one of us (A.Ö.) to be of value in three cases of increased IOP resistant to conventional treatment (Öhrström 1974). The present paper concerns the oral use of the drug in a larger clinical series.

Selection of the patients and dosage

We have given propranolol (Inderal®) in a dose of 40 to 300 mg a day (usually 80 to 160 mg) by mouth to patients with increased IOP refractory to conventio

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nal treatment or in whom such treatment was withdrawn because of side effects Existing antiglaucomatous therapy was continued except when propranolol was given to replace a drug that had to be withdrawn So far 22 patients (Table I) have been treated Response to treatment was classed I or II when the IOP was at most 22 and 26 mmHg respectively throughout the follow up period When treatment produced little or no reduction in IOP or only a transient reduction the response was considered absent

Table I
Clinical material

Disease	Cases	
Glaucoma or elevated IOP with open angle	12	
Closed angle glaucoma	4	
Secondary glaucoma	5	(following chronic uveitis 1 retinal vein thrombosis 2 uveitis secondary to discission of cataract 2)
Congenital glaucoma	1	

Results and Comments

Response was obtained in half of the cases (Table II) in which the IOP promptly fell considerably (12 ± 3 8 mmHg mean ± s d 24 hours after the beginning of the treatment) The response was classed I in four cases and II in seven cases An example of response is given in Fig 1 The cases of open angle glaucoma and primary ocular hypertension responded best Certain side effects such as insomnia and diarrhoea were observed but they were usually mild In only one case was the treatment discontinued because of side effects due to the drug

The results show that peroral administration of beta adrenergic blocking agents in adequate doses is able to produce a substantial and durable reduction of elevated IOP This reduction in IOP can be of clinical use in selected cases i e when conventional antiglaucomatous therapy fails or is not tolerated and operation is temporarily contraindicated or considered especially risky

The function of adrenergic receptors in the formation and drainage of aqueous humour is still largely unknown (Bill 19 0 Bietti 19 2 Takats et al 19,2) and theories on the mechanism of the hypotensive action of propranolol cannot

Table II

Favourable or fair response of IOP to propranolol in 11 of 22 treated patients

Sex and age		Diagnosis	Duration of the disease	Excavation of optic disc and/ or visual field defects (0 +) LE	RE	Follow up (months)	Type of response
♀	73	Wide angle glaucoma	21 years	+	+	5	I
♂	60	glaucoma	10 years	0	+	5	I
♀	65	Secondary glaucoma	4 months	0	0	3	I
♂	73	Closed angle glaucoma	3 years	0	0	3	I
♀	77	Wide angle glaucoma	7 years	+	+	5 1/2	II
♂	69	glaucoma	5 years	+	+	7	II
♂	80	glaucoma	4 years	+	+	26	II
♂	56	glaucoma	5 years	+	+	7 1/2	II
♂	55	glaucoma	8 years	–	+	4	II
♀	67	Wide angle elevated IOP	3 years	0	0	6	II
♀	68	elevated IOP	3 years	0	0	6	II

be other than speculative Better known is the action of beta adrenergic receptors on various tissues and organs of the body (Dollery et al 1969) Some of these effects must be kept in mind before giving propranolol generally

Since beta adrenergic receptors have a stimulating effect on heart activity propranolol is contraindicated in heart incompensation bradycardia or disturbances of conduction Beta receptors act also on the smooth muscle of the bronchi producing bronchodilation propranolol should therefore not be used in patients with asthma and other types of bronchospasm Another contraindication is disturbances in carbohydrate metabolism with acidosis since beta adrenergic receptors are involved in the glycogenolysis in the striate musculature and their inhibition is undesirable when liver glycogenolysis becomes insufficient (Dollery et al 1969)

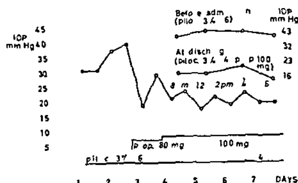


Fig 1
Favourable response to propranolol in a patient with open angle glaucoma and having intolerance for inhibitors of carbonic anhydrase Upper right diurnal tension curves before admission and at discharge

Unless contraindicated by the conditions reported above the clinical use of propranolol appears to be justified in cases of increased IOP refractory to conventional treatment

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ALIZARIN RED

Vital Staining of Cornea and Conjunctiva

BY

M S NORN

A total of 206 eyes have been subjected to vital staining with 1% alizarin red (in 64 cases followed by staining with a mixture of rose bengal and fluorescein and in 104 cases by a mixture of alizarin red and lissamine green)
Forty seven preparations have been examined in the microscope
The investigation disclosed that alizarin red *partly* effects specific staining of calcareous deposits (in the mucous thread of the conjunctival fornix in slaked lime corrosion and in meibomian gland infarcts) and *partly* causes staining resembling a weak rose bengal staining (degenerate cells dead cells and mucus)
Vital staining with the alizarin lissamine mixture gives calcium a red colour (alizarin alone) while cells and mucus are stained blue (both components) or green (lissamine alone)

Key words vital staining – cornea – conjunctiva – alizarin red – dihydroxyanthraquinone – calcium – lime

Alizarin is a red dye used for dying textiles and foodstuffs Alizarin is one of the first dyes reported to have vital staining properties Antonio Mizaldo in an encyclopaedia *from 1613 states that the bones of sheep assume a scarlet colour* after the animals have eaten the madder plant (*Rubia tinctoria*) for a few days.

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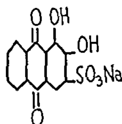


Fig 1
Formula of alizarin red

M Belchiers of London in 1736 observed the bones of pork to be red if the pig had been fed on madder (quoted *Okkels 1947*)

Alizarin has since been used for vital staining of bones in particular growing bones

Alizarin is 1 2 dihydroxyanthraquinone which is extractable from the madder plant or can be prepared synthetically Treatment with fuming sulphuric acid is employed in the preparation of alizarin red (Microme no 23) the sodium salt of dihydroxyanthraquinone 3 sulphonic acid $C_{14}H$ O SNa (Fig 1)

Alizarin red is a pH indicator the dye being yellow in acid solution of pH below 3 5 and red at pH above 5 2

Alizarin red is regarded as a fairly specific vital stain staining calcium

Fluorescein rose bengal alcian blue tetrazolium and various other dyes are used for vital staining of the cornea and conjunctiva Alizarin red does not seem to have been used before in this region. The object of the present investigation is to map out the properties of alizarin as a vital stain for cornea and conjunctiva compared with those of known vital stains

Material

A total of 0 eyes were vital stained Of these 61 were normal Among the remaining there were many cases of corneal lesions The diagnoses are seen in Table I

Table 1
206 eyes vital stained by alizarin red

Normal	61
Keratitis	24
Erosion of cornea	18
Corrosion of cornea	[illegible]
Corneal graft	7
Contact lens	10
Conj sicca	6
Infect conj	19
Simple chron conj	7
Meibomitis	7
Exophthalmus	6
Other cases	31

In addition 47 preparations were subjected to microscopy The majority of these were mucous threads removed from inferior fornices Others were scrapings from the conjunctiva or calcareous infarcts scraped off meibomian glands

Method

The eye was vital stained by 0 01 ml of 1 % alizarin red S (Gurr 1960) In 35 cases alizarin red alone was used In 64 cases this vital staining and recording of the result was followed by vital staining with a mixture of 1 % rose bengal and 1 % fluorescein Finally 104 eyes were stained by a mixture of 1 % alizarin red and 1 % lissamine green

The cornea and the conjunctiva were studied in the slit lamp and the staining results with regard to character grade and site were charted grade 3 representing moderate staining grade 2 weak staining grade 4 intense staining and grades 1 and 5 the extremes

The mixture of alizarin red and lissamine green was studied in green light (black regions representing pure red or blue mixed staining) and in white light

disclosing pure green staining not visible in green filtered light) Fluorescein staining was seen in cobalt filtered light where it showed yellow fluorescence

Alizarin red and rose bengal both stain a red colour These two dyes are therefore only comparable by successive staining

Lissamine green gives a pure green colour It can be mixed with alizarin red without causing precipitation or alteration of staining properties and is there fore directly comparable with alizarin red in mixture with this dye

Lissamine green in a 1 % concentration stains practically identically with 1 % rose bengal (Norn 1973)

In the preparations for microscopy phosphate buffer of pH 7 2 was employed under the cover slip

Microscopy

Microscopy revealed that alizarin red alone vital stains amorphous elements or fine granules found extracellularly in the mucous thread as lumps located irregularly in zones In rare cases the granules were seen arranged along mucous fibrils over short distances The red regions did not polarize nor were they arranged as vacuoles

Calcareous infarcts from meibomian glands were seen to consist of dense granular and amorphous alizarin stainable conglomerations Chalk particles and granules after slaked lime corrosion were found to be similarly stained

Alizarin may in relatively rare cases stain different cells (e g neutrophilic granulocytes nuclear squamous cells and cuboid epithelial cells) The nucleus is stained more intensely than the cytoplasm Granules in neutrophils are rarely stained

Vital staining with a mixture of alizarin red and lissamine green showed most cells to be stained green (by lissamine green) or blue (by both components) whereas very rarely red (by alizarin red alone) The red regions observed generally consisted of irregularly located zones of conglomerations or granules Meibomian infarcts were seen to consist of red conglomerations

Measurement by net ocular in the microscope gave the following average values for 24 preparations 16 % green areas 60 % blue and 18 % red For 15 normal eyes the corresponding values were 5 % green, 82 % blue and 13 % red The red zones consisted almost exclusively of cell free lumps and tracts of granules

The distribution of colours varied considerably even within the small normal material where the percentage values for red ranged from 0 to 91 those for green from 0 to 21, and those for blue from 9 to 100

The addition of 0 1 n HCl made the red areas change to a yellow colour while the subsequent addition of NaOH caused a return to the red colour

Slit Lamp

Vital staining with alizarin red *alone* rarely gave any colouring of cornea and conjunctiva The *mucous thread of the inferior fornix showed staining* in practically all cases (36/38) though most often in the form of scattered red lumps in an otherwise unstained thread

Vital staining with alizarin red *followed* by staining with a mixture of rose bengal and fluorescein showed rose bengal to stain far more often and better than alizarin Fig 2 illustrates the average grades in the different external

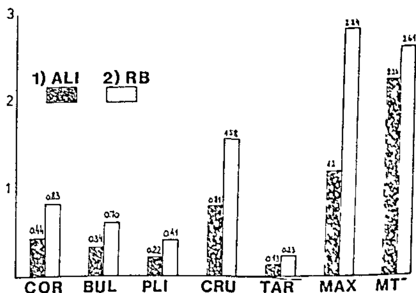


Fig 2

Vital staining with alizarin red followed by staining with a mixture of rose bengal and fluorescein Average grades in different regions 64 eyes

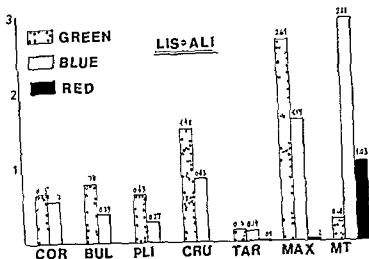


Fig 3
Vital staining with alizarin red – lissamine green mixture Average grades in different external ocular regions 104 eyes

ocular regions The average staining grade for rose bengal is seen to be twice that of alizarin on cornea, bulbar conjunctiva plica semilunaris caruncle, tarsus and Marx line The inferior fornix is not stained

Rose bengal stains more often than alizarin (Table II)

The constant relation between rose bengal and alizarin goes to show that the two dyes have the same properties but that the colour produced by 1 % alizarin is much weaker than that produced by 1 % rose bengal Fluorescein on the other hand has different properties staining for instance the cornea more often than Marx line

By vital staining with a *mixture* of alizarin red and lissamine green the latter most often stained alone (Fig 3 and Table II) A blue colour due to mixed staining was relatively frequent In rare cases a weak red colour was also seen (on Marx line and the tarsus) The fairly constant relation between lissamine green and alizarin red suggests common staining properties

Alizarin yielded a much weaker colour than lissamine green and rose bengal The latter two dyes have identical staining properties By using alizarin alone instead of in combination with rose bengal or lissamine green diagnostic data would have been missed in the following cases within the series under review

The distribution of colours varied considerably even within the small normal material where the percentage values for red ranged from 0 to 91 those for *green from 0 to 21 and those for blue from 9 to 100*

The addition of 0 1 n HCl made the red areas change to a yellow colour while the subsequent addition of NaOH caused a return to the red colour

Slit Lamp

Vital staining with alizarin red *alone* rarely gave any colouring of cornea and conjunctiva The mucous thread of the inferior fornix showed staining in practically all cases (36/38) though most often in the form of scattered red lumps in an otherwise unstained thread

Vital staining with alizarin red *followed* by staining with a mixture of rose bengal and fluorescein showed rose bengal to stain far more often and better than alizarin Fig 2 illustrates the average grades in the different external

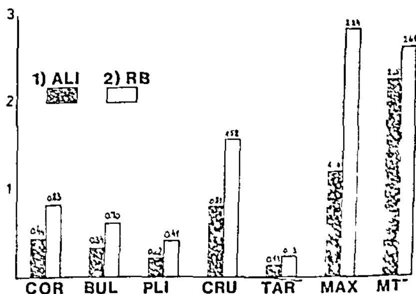


Fig 2
Vital staining with alizarin red followed by staining with a mixture of rose bengal and fluorescein Average grades in different regions 64 eyes

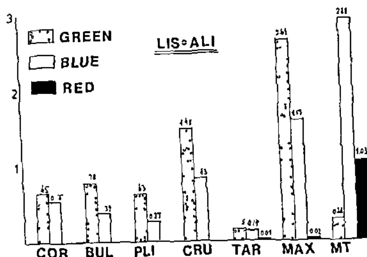


Fig 3

Vital staining with alizarin red – lissamine green mixture Average grades in different external ocular regions 104 eyes

ocular regions The average staining grade for rose bengal is seen to be twice that of alizarin on cornea bulbar conjunctiva plica semilunaris caruncle tarsus and Marx line The inferior fornix is not stained

Rose bengal stains more often than alizarin (Table II)

The constant relation between rose bengal and alizarin goes to show that the two dyes have the same properties but that the colour produced by 1 % alizarin is much weaker than that produced by 1 % rose bengal Fluorescein on the other hand has different properties staining for instance the cornea more often than Marx line

By vital staining with a *mixture* of alizarin red and lissamine green the latter most often stained alone (Fig 3 and Table II) A blue colour due to mixed staining was relatively frequent In rare cases a weak red colour was also seen (on Marx line and the tarsus) The fairly constant relation between lissamine green and alizarin red suggests common staining properties

Alizarin yielded a much weaker colour than lissamine green and rose bengal The latter two dyes have identical staining properties By using alizarin alone instead of in combination with rose bengal or lissamine green diagnostic data would have been missed in the following cases within the series under review

Table II

Number of eyes stained at the site concerned (in per cent of all examined) partly on successive vital staining with alizarin red (Ali) and then rose bengal (Rose beng) in a total of 64 eyes and partly on staining with a mixture of alizarin red and lissamine green (Ali Lis) in a total of 104 eyes

	Successive		Ali Lis mixture		
	Ali	Rose beng	Green	Blue	Red
Cornea	25	43	30	23	0
Eyeball	17	33	46	24	0
Plica	16	31	38	23	0
Caruncle	59	88	80	62	0
Tarsus	9	13	10	8	1
Marx line	70	100	96	85	2
Mucous thread	100	100	16	99	61

Marginal dendritic epidemic and central keratitis (13 out of 29) corneal degeneration corneal erosion contact lens (5 out of 10) graft keratoconjunctivitis sicca (too weak staining in all six cases)

Mucous thread in inferior fornix

Alizarin effected better staining of the mucous thread in the fornix than of the other regions The thread was practically always stained (Table II) though often only in zones The average grade on successive staining with rose bengal was almost identical with that for rose bengal alone (Fig 2)

On vital staining with an alizarin lissamine mixture (Fig 3) mixed staining and pure alizarin staining predominated indicating that alizarin stains other elements besides those stained by rose bengal and lissamine green

A red dye in mixture with lissamine green disclosed components that were not stained by lissamine green i e were neither degenerate nor dead cells Such specific red staining of the mucous thread is most frequently seen in keratoconjunctivitis sicca and perhaps also in slaked lime corrosion corneal degeneration and grafting

Meibomian gland infarct

A total of 83 infarcts were examined 42 % of which were vital stained (8 % by lissamine alone 34 % by alizarin and another dye) In most cases there was intense staining of the central part of the infarct

In a case of slaked lime corrosion necrotic tissue was found at the lid margin This was only stained by alizarin and not by lissamine

Discussion

The present investigation showed that alizarin has the same vital staining properties as rose bengal and lissamine green i e it stains degenerate cells dead cells and mucus

However alizarin yields a much weaker colour than rose bengal and lissamine green It is thus referable to the series of weak vital stains eosin merbromine scarlet red fuchsin brilliant cresyl blue methylene blue trypan blue and bromothymol blue (Norn 1972)

In addition alizarin has special properties which distinguishes it from the above mentioned vital stains It stains conglomerations in the mucous thread of the conjunctival fornix the contents of meibomian gland infarcts and necrosed tissue after slaked lime corrosion This staining may be specific of alizarin red the lissamine green component of the mixture possibly not staining the region concerned Alizarin red must be supposed to stain specifically calcareous deposits

A meibomian gland (tarsal gland) infarct can be regarded as fat retained in the gland Lime salts may in some cases become deposited in these hard masses (Duke Elder 1940)

In cases of pronounced retention the region will project The epithelium may become damaged with consequent punctate staining by lissamine green and rose bengal

In some cases the calcified fatty mass penetrates through the epithelium In such cases intense specific alizarin staining is seen in these conglomerations

To summarize we may conclude that alizarin stains calcareous deposits and in addition causes weak staining of the same elements as rose bengal i e a few degenerate and dead cells

Calcareous deposits may be stained red by a mixture of alizarin and lissamine, whereas degenerate cells dead cells and mucus are stained by both components (blue) or by lissamine alone (green)

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GLUCOSE PYRUVATE AND CITRATE CONCENTRATIONS IN THE AQUEOUS HUMOUR OF HUMAN CATARACTOUS EYES

BY

A BRUUN LAURSEN and S E LORENTZEN

Results are reported of determinations of glucose concentrations in aqueous humour and plasma of pyruvate concentrations in aqueous humour and of citrate concentrations in aqueous humour and plasma from 22 non fasting and 17 fasting patients with senile cataracts five fasting diabetics with senile cataracts and four patients with cataract and other ocular diseases Higher pyruvate concentrations were found in the non fasting group with senile cataract than in the fasting senile cataract group ($P < 0.01$) The group of diabetics likewise presented higher pyruvate concentrations than the group of fasting senile cataractous patients ($P < 0.05$)
The Spearman coefficient of rank correlation showed a positive correlation between cataract density and citrate concentrations in the aggregate series ($P < 0.05$) Senile cortical cataracts and senile nuclear plus cortical cataracts differed in that the citrate aqueous/citrate plasma ratio calculated for the whole series collectively was the lowest in the latter group ($P < 0.05$) Further investigations into the metabolite concentrations at different stages of cataract are however required as an error of the first kind because of multiple significance tests cannot be left out of account in the present material

Key words glucose – pyruvate – citrate – aqueous humour – cataracts senile human

Received March 1 1974

In two previous papers the results are stated of determinations of pyruvate and citrate concentrations in aqueous humour from oxen (Bruun Laursen 1972) and rabbits (Bruun Laursen 1973) In both animal species it was demonstrated that the pyruvate/citrate ratio fell with increasing age

In the present paper the results are reported of determinations of glucose pyruvate and citrate concentrations in aqueous humour from patients with senile cataracts

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Material

Samples of aqueous humour were drawn from 22 non fasting and 17 fasting patients with senile cataract five fasting diabetics with senile cataract and four patients with both cataract and other ocular diseases The fasting patients had been allowed no food since the evening before the operation The non fasting patients had eaten a doubtless fairly high carbohydrate breakfast

No aqueous humour from normal human eyes has been procurable so far

The aqueous humour samples were aspirated during cataract operation just before the anterior chamber was opened A puncture was made through the limbus corneae with a fine subcutaneous cannula the eye being under local and retrobulbar anaesthesia The operations were performed between 8 and 11 a m The samples (volumes ranging from about 70 to about 200 μl) were promptly heated to opalescence to destroy enzymes and immediately after submitted to analysis for glucose followed by freezing to −18 C The samples from the non fasting patients were not analyzed for glucose till after freezing just before the *pyruvate and citrate determinations which were 3–6 hours after aspiration*

The blood sugar level was determined on blood from the auricular capillaries which was analyzed immediately For plasma citrate determinations brachial vein blood was used which like the auricular capillary blood was withdrawn just before the cataract operation The venous blood was then promptly centrifuged and the plasma left in the refrigerator (at +4 C) until the analyses were to be performed

Some of the patients with senile cataract also suffered from the circulatory and respiratory diseases common to this age group

The results from the group of eyes with cataract and other associated ocular diseases have not been included in the statistical calculations

Methods

The glucose concentrations were determined by means of a Beckmann glucose analyzer the pyruvate and citrate concentrations by a modification of Moellering & Gruber s enzymatic spectrophotometric method (Bruun Laursen 1973)

Results

The results have been set out in Table I Tables II and III show the results of statistical calculations

Significantly higher pyruvate concentrations were found in aqueous humour samples from 22 non fasting patients with senile cataract (median 210 μmol/l) and five fasting diabetics with senile cataract (median 199 μmol/l) than in samples from 17 fasting patients with senile cataract (median 152 μmol/l) No significant differences were demonstrated between the glucose concentrations in the aqueous humour of the above groups (medians 3 9 4 3 and 3 1 mmol/l respectively)

Neither did the three stated groups differ significantly with regard to aqueous humour citrate concentrations (93 98 and 112 μmol/ l respectively)

The pyruvate/citrate ratio was significantly higher in aqueous humour from non fasting patients with senile cataract (1 9[illegible]) than in aqueous humour from the group of fasting patients with senile cataract (1 04)

A positive correlation was found between cataract density and citrate concentration both in the group of fasting patients with senile cataract separately ($P < 0\ 05$) and in the whole series of fasting and non fasting patients with senile cataract (diabetics excepted) see Table III

Further in 18 non fasting patients a positive correlation was noticed between the site of the cataract and the ratio of citrate in aqueous/citrate in plasma ($P < 0\ 05$ – diabetics excepted)

For classification of cataract sites the following system was applied 1 cortical 2 nuclear 3 cortical and nuclear Density of cataract was graduated in the following manner 1 *Sl t lamp* mil l immature cataract with regard to extension and/or density *Ophtl lm copy* l ghtly to moderately blurred insight pre operatively 2 *Slit lamp* pronounced immature cataract *Ophthalmoscopy* details of the fundus are seen with d fficulty or not at all pre operatively 3 *Sl t lamp* mature or almost mature cataract *Ophtl lmoscopy* grey or white reflex No insight

Table I

Data on glucose pyruvate and citrate concentrations in aqueous humour from eyes with senile cataracts For graduation of cataract density and sites see p 000

Fasting pts with senile cataract

No	Age years	Cataract		Pyr aq	Citr aq	Citr pl	Gluc aq	Gluc pl	Ratio Gluc aq / Gluc pl	Ratio Gluc aq / Pyr aq	Ratio Pyr aq / Citr aq	Ratio Citr aq / Citr pl	Ratio Gluc aq / Citr aq
		Density	Site	μmol/l			mmol/l						
1	51	1	1	201	87		3 6	5 7	0 6	17 9	2 31		41 4
2	51	3	3	225	105		3 8	4 7	0 8	16 9	2 14		36 2
3	53	1	2	173	124		2 8	5 1	0 5	16 2	1 40		22 6
4	56	3	3	82	102		4 2	6 0	0 7	51 2	0 80		41 2
5	58	3	3	173	183		3 0	5 2	0 6	17 3	0 95		16 4
6	67	3	3	114	128		1 8	4 7	0 4	15 8	0 89		14 1
7	69	2	3	145	96		2 8	5.5	0 5	19 3	1 51		29 2
8	72	2	3	82	94		3 1	5 2	0 6	37 8	0 87		33 0
9	74	3	3	106	177	115	3 2	5 0	0 6	30 2	0 60	1 54	18 1
10	74	2	1	167	170		5 4	6 8	0 8	32 3	0 98		31 8
11	77	3	3	99	166	118	3 0	6 6	0 5	30 3	0 60	1 41	18 1
12	79	2	1	89	87		3 3	5 7	0 6	37 1	1 02		37 9
13	51	1	3	162	85		3 7	5 0	0 7	22 8	1 91		43 5
14	83	3	3	136	104		2 6	5 0	0 5	19 1	1 31		25 0
15	85	3	3	157	151		2 5	5 8	0 4	15 9	1 04		16 6
16	85	3	3	152	112	202	3 4	6 9	0.5	22 4	1.36	0 55	30 4
17	86	2	3	179	124		3 1	5 8	0 5	17 3	1 44		25 0
Median	74	3	3	152	112	118	3 1	5 5	0 6	19 3	1 04	1 41	29 2
10 % Lerc	51	1	1	82	87		2 4	4 7	0 4	15 9	0 60		15 9
90 % Lerc	85	3	3	206	178		4 4	6 8	0 8	40 [illegible]	2 16		41 8
Range of variation	3	2	2	141	95	87	3 6	2 2	0 4	[illegible] 4	1 71	[illegible]	23 4

Non fasting pt with senile cataract													
18	53	3	3	25	93			6 4			2 72		
19	58	1	1	193	134	167					1 44	0 80	
20	58	1	3	254	85	65					2 99	1 31	
21	61	2	3	187	91	89					2 06	1 11	
22	61	1	3	220	127						1 73		
23	6	1	2	206	92	95	2 7			13 1	2 24	0 97	29 4
24	62	2	3	275	132		3 9	5 4	0 7	14 2	2 08		29 6
25	67	2	1	229	93	94	4 1	5 7	0 7	17 9	2 46	0 99	44 1
26	68	3	3	212	72	89	4 7	6 8	0 7	22 9	2 95	0 81	65 3
27	69	1	3	194	83	106					2 34	0 73	
28	71	1	3	210	118	67	4 0	6 8	0 6	19 1	1 78	1 76	33 9
29	74	2	3	147	112	102		6 8			1 31	1 10	
30	74	1	1	192	74		4 0	8 2	0 5	20 8	2 60		54 1
31	75	2	1	102	77	160					1 33	0 48	
32	76	3	3	145	150	115	3 4	5 6	0 6	29 5	0 97	1 30	22 7
33	77	3	3	82	66	108					1 24	0 61	
34	77	3	3	257	185	138					1 38	1 36	
35	79	2	1	140	75	105	3 8	7 0	0 5	27 1	1 87	0 71	50 7
36	81	2	1	210	177	271					1 19	0 65	
37	82	3	3	249	142	149	3 5	5 9	0 6	14 1	1 75	0 95	24 7
38	83	2	3	248	89	103					2 79	0 85	
39	85	1	1	236	120	152	2 7	4 5	0 6	11 4	1 97	0 77	22 5
Median	72 5	2	3	210	93	106	3 9	6 4	0 6	18 5	1 92	0 90	31 2
10 % l erc	58	1	1	113	73	67	2 7	4 7	0 5	11 6	1 21	0 60	22 5
90 % l erc	84	3	3	256	169	177	4 6	8 0	0 7	26 7	2 90	1 40	64 2
Range of variation	32	2	2	193	122	206	2 0	3 7	0 2	15 7	2 02	1 98	42 8

Table 1

Data on glucose pyruvate and citrate concentrations in aqueous humour from eyes with senile cataracts For graduation of cataract density and sites see p 000

Fasting pts with senile cataract

No	Age years	Cataract Density	Cataract Site	Pyr aq (µmol/l)	Citr aq (µmol/l)	Citr pl (µmol/l)	Gluc aq (mmol/l)	Gluc pl (mmol/l)	Ratio Gluc aq / Gluc pl	Ratio Gluc aq / Pyr aq	Ratio Pyr aq / Citr aq	Ratio Citr aq / Citr pl	Ratio Gluc aq / Citr aq
1	51	1	1	201	87		3 6	5 7	0 6	17 9	2 31		41 4
2	51	3	3	225	105		3 8	4 7	0 8	16 9	2 14		36 2
3	53	1	2	173	124		2 8	5 1	0 5	16 2	1 40		22 6
4	56	3	3	82	102		4 2	6 0	0 7	51 2	0 80		41 2
5	58	3	3	173	18		3 0	5 2	0 6	17 3	0 95		16 4
6	67	3	3	114	128		1 8	4 7	0 4	15 8	0 89		14 1
7	69	2	3	145	96		2 8	5 5	0 5	19 3	1 51		29 2
8	72	2	3	82	94		3 1	5 2	0 6	37 8	0 87		33 0
9	74	3	3	106	177	115	3 2	5 0	0 6	30 2	0 60	1 54	18 1
10	71	2	1	167	170		5 4	6 8	0 8	32 3	0 98		31 8
11	77	3	3	93	166	118	3 0	6 6	0 5	30 3	0 60	1 41	18 1
12	79	[illegible]	1	89	87		3 3	5 7	0 6	37 1	1 02		37 9
13	81	1	3	162	85		3 7	5 0	0 7	22 8	1 91		43 5
14	83	3	3	136	104		2 6	5 0	0 5	19 1	1 31		25 0
15	85	3	3	157	151		2.5	5 8	0 4	15 9	1 04		16 6
16	85	3	3	152	112	202	3 4	6 9	0 5	22 4	1 36	0 55	30 4
17	86	2	3	179	121		3 1	5 8	0 5	17 3	1 44		25 0
Median	74	3	3	152	112	118	3 1	5 5	0 6	19 3	1 01	1 41	29 2
10% Perc	[illegible]	1	1	82	87		2 4	4 7	0 4	15 [illegible]	0 [illegible]		1[illegible]
90% Perc	8[illegible]	3	3	20[illegible]	178		4 4	[illegible] 8	0 8	40 [illegible]	2 1[illegible]		41 8
Range of variation	3[illegible]	2	2	141	9[illegible]	87	3[illegible]	2 2	0 4	[illegible] 4	1 71	0 [illegible]	2[illegible]

Aque us hum ur from ame eye bef re cataract extraction (non fasting) and after cataract extraction (fasting) The pt also had glaucoma retinal detachment and angiomatosis of retina

4													
Bef re cat extract	53	3	3	9 1	114		3 0	5	0 6	12 0	2 22		?6 3
after cat ext	53	0	0	179	104		2 8	4 4	0 6	15 6	1 72		26 9

Cataract in imbecile with pigmented naevi of iris (non fasting)

46	60	3	3	0	132	91					0	1 4?	

Co nplicated cataract – chronic uveitis and sinusitis (non fasting)

47	31	3	3	175	67	61					2 61	1 10	

Cataract in pt with simplex glaucoma and iridic naevi (non fasting)

48	81	2	1	226	117	146					1 93	0 80	

Table I (cont.)

Fasting and non fasting patients with senile cataract

No	Age years	Cataract Density	Cataract Site	Pyr aq (µmol/l)	Citr aq (µmol/l)	Citr pl (µmol/l)	Gluc aq (mmol/l)	Gluc pl (mmol/l)	Ratio Gluc aq / Gluc pl	Ratio Gluc aq / Pyr aq	Ratio Pyr aq / Citr aq	Ratio Citr aq / Citr pl	Ratio Gluc aq / Citr aq
Median	74	2	3	179	105	108	3 4	5 7	0 6	19 1	1 44	0 93	29 6
10 % Perc	53	1	1	89	75	70	2 6	4 7	0 5	13 9	0 87	0 57	16 6
90 % Perc	85	3	3	252	177	195	4 3	6 9	0 7	37 2	2 72	1 40	51 4
Range of variation	35	2	2	193	122	206	3 6	3 7	0 4	39 8	2 39	1 28	51 2

Fasting diabetics with senile cataract

No	Age years	Cataract Density	Cataract Site	Pyr aq (µmol/l)	Citr aq (µmol/l)	Citr pl (µmol/l)	Gluc aq (mmol/l)	Gluc pl (mmol/l)	Ratio Gluc aq / Gluc pl	Ratio Gluc aq / Pyr aq	Ratio Pyr aq / Citr aq	Ratio Citr aq / Citr pl	Ratio Gluc aq / Citr aq
40	70	3	3	199	73	123	5 2	7 3	0 7	26 1	2 73	0.59	71 2
41	72	3	3	175	94		3 0	5 0	0 6	17 1	1 86		31 9
42	79	2	3	221	102		6 2	11 3	0 5	28 1	2 17		60 8
43	86	3	3	136			4 2	6 0	0 7	31 6			
44	80	3	3	229	137	147	2 9	4 3	0 7	12 6	1 67	0 93	21 8
Median	79	3	3	193	98	135	4 3	6 0	0 7	26 1	2 02	0 76	46 4
25 % Perc	71			156	78		3 0	4 7	0 6	14 9	1 72		23 9
75 % Perc	83			225	130		5 7	9 3	0 7	29 9	2 62		63 1
Range of variation	16	1	0	33	44	24	3 3	7 0	0 2	13 0	1 06	0.34	50 0

Table III

P values for correlation between x and y values found through the Spearman correlation coefficient and where the sites are concerned by the Wilcoxon test for two samples

For graduation of cataract density and sites see Results p 479

	Dependence of	Gluc aq	Pyr aq	Citr aq	Gluc aq / Gluc pl	Gluc aq / Pyr aq	Pyr aq / Citr aq	Citr aq / Citr pl	Gluc aq / Citr aq
n		17	17	17	17	17	17		17
Fasting pts Senile cat	age density	> 0 10 > 0 10	> 0 10 > 0 10	> 0 10 < 0 05	> 0 10 > 0 10	> 0 10 > 0 10	> 0 10 > 0 10		> 0 10 > 0 10
n =		10	22	22	[illegible]	10	22	18	10
Non fasting patients Senile cat	age density compared with site 3	> 0 10 > 0 10 > 0 10	> 0 10 > 0 10 > 0 05	> 0 10 > 0 10 > 0 10	> 0 10 > 0 10 > 0 10	> 0 10 > 0 10 > 0 10	> 0 10 > 0 10 > 0 10	> 0 10 > 0 10 < 0 05	> 0 10 > 0 10 > 0 10
n =		27	30	30	26	27	39	21	27
Fasting and non fasting patients Senile cat	density site 1 compared with site 3	> 0 10	> 0 10	0 05 > 0 10	> 0 10	> 0 10	> 0 10	< 0 05	< 0 05
n =		5	5		5	5			
Fasting diabetic pts Senile cat	age density	> 0 10 > 0 10	> 0 10 > 0 10		> 0 10 > 0 10	> 0 10 > 0 10			

Table II

Comparison between fasting and non fasting patients with senile cataracts and fasting diabetics with senile cataracts P values found by the Wilcoxon test for two samples

Senile cataracts fasting	Senile cataracts – non fasting: Gluc aq	Pyr aq	Citr aq	Gluc aq / Gluc pl	Gluc aq / Pyr aq	Pyr aq / Citr aq	Gluc aq / Citr aq
Gluc aq	> 0 10 / > 0 10						
Pyr aq		< 0 01 / < 0 05					
Citr aq			> 0 10 / > 0 10				
Gluc aq / Gluc pl				> 0 10 / > 0 10			
Gluc aq / Pyr aq					> 0 10 / > 0 10		
Pyr aq / Citr aq						< 0 01 / < 0 05	
Gluc aq / Citr aq							> 0 10 / > 0 10

Senile cataracts in fasting diabetics

In other words the present material showed the citrate concentration to rise with increasing cataract density and the ratio of citrate in aqueous to citrate in plasma to be higher when both cortex and nucleus were involved than when the cortex alone was affected For the series as a whole (the diabetics excepted) a significantly lower glucose aqueous/citrate aqueous ratio was noticed in the group of patients with both nuclear and cortical cataract than in the group with cortical cataract alone ($P < 0\ 05$)

more accounts for the wide ranges of variation which must be supposed in the great majority of cases to be due to real interindividual variations These were also found in rabbits where in addition marked intraindividual fluctuations of pyruvate and citrate concentrations were noticed (Bruun Laursen 1973)

Nuclear and cortical cataracts show differences in the chemical changes occurring in connection with cataract development (Maraini & Mangili 1973) The classification on clinical grounds into nuclear and cortical types used in the present paper thus seems to be consistent with differences in the chemical patho logy of cataract

In the series under review we found higher pyruvate concentrations in non fasting patients with senile cataracts and fasting diabetics with senile cataracts than in other fasting patients with senile cataracts though the glucose concen trations did not differ significantly in the stated groups The glucose medians (3 9 mmol/l in non fasting patients and 4 3 mmol/l in fasting diabetics with senile cataracts against 3 1 mmol/l in the group of fasting patients with senile cataracts) in association with an unaltered glucose aqueous/pyruvate aqueous ratio between the fasting group with senile cataract on one hand and the non fasting group with senile cataract and the diabetics on the other nevertheless suggest that the pyruvate concentration changes bear relation to corresponding changes of the much higher glucose concentrations in the aqueous humour

In a patient with a pathological eye (glaucoma retinal detachment angioma tosis of retina) we noticed before cataract extraction (non fasting?) and after cataract extraction (fasting) concentrations and ratios corresponding to those found in other non fasting and fasting patients respectively with senile cataract Pohjola (1966) found no significant difference between the glucose concentra tions in aqueous humour samples aspirated from the same eye before and after cataract extraction In the present series four patients belonging to the group with cataract and other ocular diseases showed no deviations from the conditions in the group with senile cataract alone as regards concentrations of glucose pyruvate and citrate except that an imbecile patient with iridic naevi apparent ly had no pyruvate in the aqueous humour

The age groups included in the present investigation showed no correlation between age and concentrations of glucose, pyruvate and citrate or between age and ratios of glucose aqueous/glucose plasma glucose aqueous/pyruvate aqueous pyruvate aqueous/citrate aqueous citrate aqueous/citrate plasma, or glucose aqueous/citrate aqueous In oxen and rabbits where a larger proportion of the normal span of life including part of the growth period was represented the pyruvate citrate ratio was found to decline with increasing age (Bruun Laursen 1972 and Bruun Laursen 1973)

It is difficult to say whether the same is the case in normal human eyes Even

Discussion

The glucose values in the present investigation are in accordance with those noted by Pohjola (1966) on the basis of 62 samples of normal human aqueous humour 65–86 mg % (3.6–4.8 mmol/l) plasma glucose level 85–118 mg % (4.7–6.6 mmol/l) and ratio of glucose in aqueous to glucose in plasma 0.,6–0.63 falling with increasing age within the interval of 20–80 years Pohjola found no significant difference between the glucose concentrations in aqueous humour from 20 eyes with senile cataract and those in aqueous humour from normal eyes of about the same age group

The values arrived at by de Berardinis et al (1965) in aqueous humour from eyes with different forms of cataract (fasting – non fasting?) 2.42 mmol/kg H_2O ± 0.80 (mean ± s d) were somewhat lower than in the present series In aqueous humour from eyes with clear lenses but affected by other ocular diseases the corresponding values were 3.00 mmol/kg H_2O ± 2.04 These workers employed a glucose oxidase method for the glucose determinations but no information is available concerning glycolysis inhibiting measures within the period between the aspiration of aqueous humour and the glucose determinations

Citrate concentrations in aqueous humour from eyes with senile cataract have been determined by Gronvall (1937) who employed Thunberg s technique Using methylene blue as indicator he found 25.65 μg/ml ± 0.67 (mean ± SEM) in 53 subjects corresponding to 134 μmol/l Using indigotrisulphonate as indicator he found 16.35 μg/ml ± 0.58 in 21 subjects (85 μmol/l) He noticed that one patient with basal cell cancer of the conjunctiva but an otherwise normal eye had an aqueous humour citrate concentration equal to those in aqueous humour samples from senile cataractous eyes

The results of the present investigation are in fair agreement with those achieved by Gronvall with indigotrisulphate as indicator

No reports have been found in the literature on pyruvate concentrations in aqueous humour from human cataractous eyes

Reddy & Kinsey (1960) among others found somewhat higher glucose concentrations in posterior than in anterior aqueous humour from rabbits If similar conditions prevail with regard to glucose pyruvate and citrate in humans the relatively wide ranges of variation in the present material may perhaps be due to differences in the proportional volumes of anterior and posterior aqueous humour in the aspirated samples The chambers were as far as possible emptied of aqueous Glycolysis on the other hand can be left out of account as a possible cause of the ranges of variation owing to the treatment given to the material (Bruun Laursen 1973)

Though a certain analytical uncertainty exists (Bruun Laursen 1973) this no

Maraini G & Mangili R (1973) Differences in proteins and in the water balance of the lens in nuclear and cortical types of senile cataract In Ciba Foundation Symposium 19 (new series) *The Human Lens in Relation to Cataract* pp 79–97 Associated Scientific Publishers Amsterdam

Pohjola, S (1966) The glucose content of the aqueous humour in man. *Acta ophthal (Kbh) Suppl 88*

Reddy D V N & Kinsey V E (1960) Composition of the vitreous humour in relation to that of plasma and aqueous humours *Arch Ophthal 63* 715–720

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if this were so the age interval between the patients examined is doubtless too small to allow for the conclusion that absence of a correlation between age and pyruvate/citrate ratio indicates an essential difference between the aqueous humour in normal eyes and that in cataractous eyes

The observed rising citrate concentrations in the aqueous humour and the observed rising citrate aqueous/citrate plasma ratio in relation to increasing cataract density and to combined cortical and nuclear cataract compared with cortical cataract alone suggest alterations of the energy metabolism in connection with the development of cataract The glucose and pyruvate concentrations being independent of these alterations a selective change of permeability of citrate must be presumed if a change of permeability is to be regarded as the sole explanation of the alterations observed However so many significance tests have been carried out that chance *significances on the 5 %* level must be expected Further investigations are therefore required before we can decide whether the development of cataract is attended by alterations of the concentrations of certain energy metabolic products in the aqueous humour

Acknowledgments

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Gronvall H (1937) Citric acid studies referring to the eye *Acta ophthal (Kbh) Suppl 14*

Harwerth & Sperling (1971) described prolonged colour blindness induced by intense spectral lights in specially trained monkeys By the two colour threshold technique of Stiles (1949 1959) where the sensitivity is measured during constant chromatic adaptation seven typical response patterns could be referred to three separate cone mechanisms Wald (1964) on the basis of Stiles principle devel oped a procedure for measuring spectral sensitivity of three groups of cones in human beings By this technique the eye was constantly adapted to a small high ly illuminated field seen in the Maxwellian view A similar technique was used by Marré (1969)

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The purpose of the present work is to describe a method which can be applied in clinical evaluation of separate receptor functions by central as well as by peripheral localizations The basic idea of the method is to combine static colour perimetry with the two-colour threshold technique of Stiles also to study some typical response patterns obtained in normal persons

Method

As the ordinary background illumination of the perimeter has proved too weak when coloured filters are being inserted it has been eliminated and replaced by the light from a projector The light beam is directed from above the head of the patient to the centre of the sphere where an oval field is illuminated The field comprises about 30° in the largest horizontal diameter The projector is a Rolley with 150 watt halogen lamp and &85 lens The current supply is taken from a constant voltage transformer Smaller adjustments are made with a rotary transformer in order to obtain a constant luminosity level in the field The following coloured filters are used, inserted as slides Wratten filter no 4 B transmitting wave lengths below 490 nm giving a blue light Wratten filter no 35 transmitting wave lengths below 465 nm and above 650 nm giving a purple light and Wratten filter no 22 with transmission of wave lengths longer than 555 nm giving a yellow light No 4 B is used in a double layer and no 22 in a 4 double layer The illuminance is measured by a luxmeter (EBLX3 Hartmann & Braun) at right angles to the light beam with the same distance from the apparatus to the centre of the sphere The standard setting is 37 lux for filter no 4/B 18 lux for filter no 35 and 900 lux for filter no 2. Taking 0 7 as the reflexion factor of the perimeter the calcula ted values of the luminance in the field is 6 asb 55 asb and 2030 asb for the filters no 4 B no 35 and no respectively

The University Eye Clinic Rikshospitalet Oslo Norway
(Head Professor Thore Lie Thomassen M D)

THE COLOUR RECEPTORS STUDIED BY INCREMENT THRESHOLD MEASUREMENTS DURING CHROMATIC ADAPTATION IN THE GOLDMANN PERIMETER

BY

EGILL HANSEN

With the help of a method based on the Goldmann projection perimeter measurements of spectral sensitivity during chromatic adaptation are performed utilizing the principle of the two colour threshold technique of Stiles The method is flexible and is used for central as well as for peripheral registration Characteristic sensitivity curves and static perimetry curves of normally sighted persons are demonstrated showing that each type of cones can be clearly separated and identified With smaller sized objects the foveal blue blindness is confirmed whilst in the parafoveal region the response of the blue receptor is good

Key words colour perception – increment thresholds – Goldmann perimeter – chromatic adaptation – spectral sensitivity – cone mechanisms – foveal blue blindness

The colour receptors can be influenced separately by adaptation to coloured lights of certain qualities As early as 1898 Burch described artificial temporary colour blindness in normally sighted persons after exposure to coloured lights of high intensity Brindley (1953) described the changes in his personal colour vision after illuminating the retina with strong lights of various qualities

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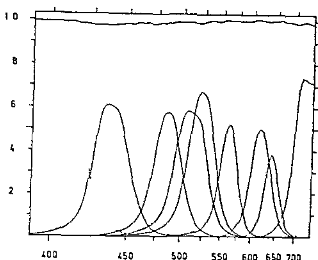


Fig 1
The transmission of the interference filters The abscissa indicates the wave lengths (nm) and the ordinate the transmission

A collection of nine changeable interference filters is used the filters being attached to the projection arm of the perimeter The transmission properties of the filters are shown in Fig 1 The half band widths vary between 30 and 47 nm The energy transmission for each filter has been calculated on the basis of the luminous flux of the perimeter lamp specified by Verriest & Israel (1965) The initial value is set at 1000 asb for the largest white object of greatest intensity (object V) Object IV is the standard object used during the examinations Its noted size is 16 mm² with the largest diameter comprising 1° 3 It is important that relatively new lamps are used The energy transmitted through the interference filters are equivalated by introducing a factor k which is calculated for each filter The sensitivity corresponding to the λ_{max} of the filters is determined by adding log k to the increment threshold value which has been found for the particular filter The threshold value is expressed as Density i e the density of the grey filter step corresponding to the scale value (density = log 1 transmission)

Procedure

The examination is undertaken with the one eye occluded There is an adaptation period of 5 min in total darkness followed by 5 min with the actual background illumination before beginning the registrations For the central registration the patient is told to fixate between the opposite points of two dark triangles attached to the sphere The dark fixation spot of the perimeter is occluded with white tape in order to avoid after contrast effects The light stimuli are presented in the same manner as for ordinary

static perimetry The duration of the stimuli is about 1 sec and the period between the stimuli about 3 to 5 sec The final threshold value is the result of several trials with varying intensities from weaker to stronger values The patient is instructed to notice when the object light is just visible and to disregard colours The examination is started and finished by threshold determination of the white stimulus light The two values should be equal or close to each other Otherwise the examination is repeated completely or partly Correction for ametropy or presbyopy is done according to the rules for ordi nary perimetry An artificial pupil of 3 mm is used for the central registrations in coloured backgrounds but all the peripheral registrations are done with normally sized pupils Registration begins centrally after which peripheral registrations may follow or static perimetry may take place with one of the interference filters

Material

All the persons examined proved to have normal colour vision after examination with pseudo isochromatic charts Their general ophthalmological status has been found nor mal Men only were examined in order to exclude the possibility of heterozygots for colour vision deficiencies Their age group was between 21 and 41 years The visual acuity on the eye tested was 6/6 with or without correction Greater refractive errors have been avoided

Results

A certain chromatic adaptation may be traced even in the standard white illu mination of the perimeter as shown in Fig 2 (bottom curve) This may be attributed to the quality of the white background light which has an unpro portionately greater effect in the long wave length region than in the shorter wave lengths Consequently there is a great difference in the bleaching effect upon the three types of cones As it is the same light source that provides the background light and the stimulus light and both of them are distorted in the same proportion as regards the radiant energy it seems reasonable to discount the energy distribution of the perimeter lamp when calculating the spectral sensitivity Such a compensated sensitivity curve is shown in the upper part of Fig 2 However some degree of predominance can be traced in the long wave length region

Purposely distorted sensitivity curves are obtained with the Wratten colour filters Fig 3 shows the spectral sensitivity during complete adaptation to the blue light and Fig 4 to the purple light Both curves show a broad maximum The former which is dominated by the red sensitive receptors has a maximum between 5?2 and 61? nm and the latter which is dominated by the green sensi

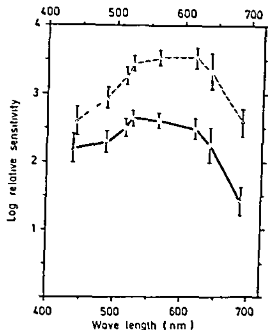


Fig 2

Relative spectral sensitivity in the standard white background light (31.5 asb) for 5 normal individuals The variation (± 1 s d) is indicated by vertical lines The bottom curve shows the sensitivity calculated with allowance for the energy distribution of the lamp The upper curve shows the spectral sensitivity without corrections for the lamp The curve is displaced upwards 1 log unit

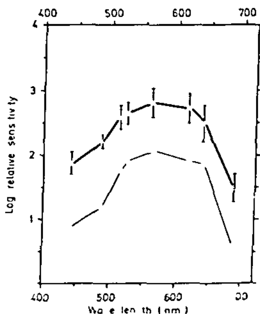


Fig 3

Relative spectral sensitivity of 5 normal individuals in blue background light (Wratten filter no 47B 37 lux) The variation (± 1 s d) is indicated by vertical lines The lower curve shows the sensitivity for the smallest object size (1 16 mm) in one of the normal persons by central registration

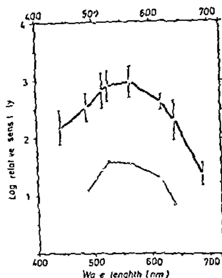


Fig 4

Relative spectral sensitivity of 5 normal individuals in purple background light (Wratten *filter no 3.. 78 lux)* The *variation (± 1 s d) is indicated by vertical lines* The *lower* curve shows the central sensitivity for the smallest object (1/16 mm²) in one of the examinees

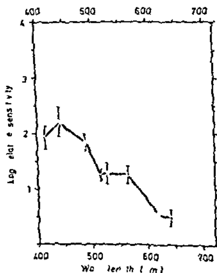


Fig 5

Relative spectral sensitivity for 5 normal individuals in yellow background light (Wratten filter no .. 2900 lux) The variation (± 1 s d.) *is indicated by vertical lines.*

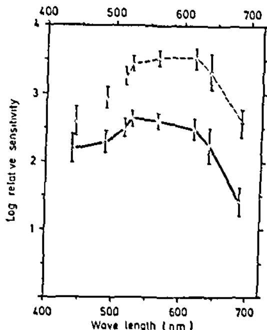


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Relative spectral sensitivity in the standard white background light (31 5 asb) for 5 normal individuals The variation (± 1 s d) is indicated by vertical lines The bottom curve shows the sensitivity calculated with allowance for the energy distribution of the lamp The upper curve shows the spectral sensitivity without corrections for the lamp The curve is displaced upwards 1 log unit

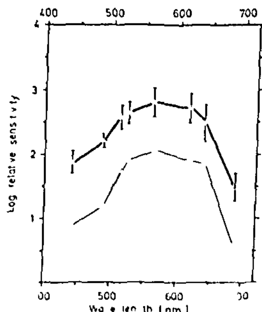


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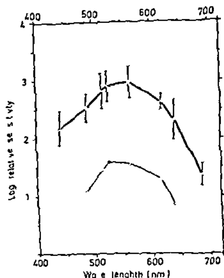


Fig 4

Relative spectral sensitivity of 5 normal individuals in purple background light (Wratten filter no 35 8 lux) The variation (± 1 s d) is indicated by vertical lines The lower curve shows the central sensitivity for the smallest object (1/16 mm²) in one of the examinees

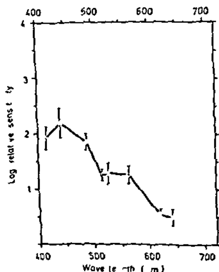


Fig 5

Relative spectral sensitivity for 5 normal individuals in yellow background l ght (Wratten filter no ? 900 lux) The variation (± 1 s d.) is indicated by vertical lines

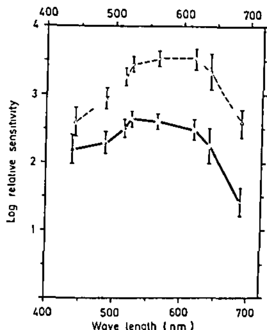


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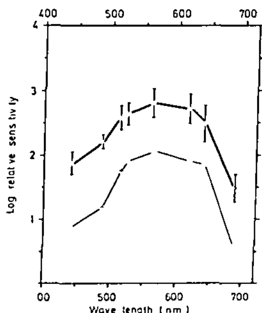


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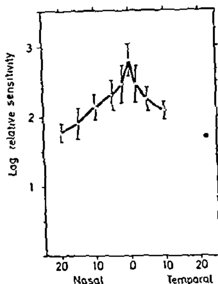


Fig 7

Static perimeter curve obtained in blue background with a red object light (λ_{max} = 617 nm) The curve shows the average value of 5 normal individuals The vertical lines indicate the variation (± 1 s d)

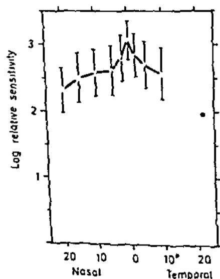


Fig 8

Static perimeter curve in purple background with a yellow green object light (λ_m = 56 nm) The curve shows the average value of 5 normal individuals The vertical lines indicate the variation (± 1 s d)

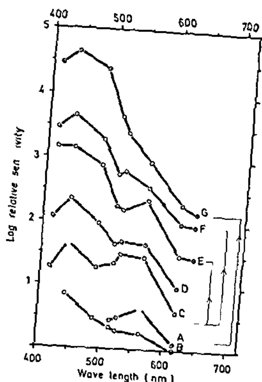


Fig 6

Relative spectral sensitivity with different object sizes in yellow background lights of varying intensity Curve A shows the sensitivity for the smallest object (1/16 mm²) by central registration and curve B for the same object at 2° to the nasal side Curve C indicates the central sensitivity for object II (1 mm) and curve D for object IV (16 mm) all the curves being registered in moderate illuminance 1500 lux Curve E is obtained with object IV in greater illuminance 2900 lux and curve F with the same object in a background light of still higher intensity 4500 lux Curve G is registered at 10° to the nasal side with the illuminance of 2900 lux

tive receptors has a maximum between 525 and 562 nm With the bleaching yellow light a more distinct top appears at 441 nm corresponding to the blue receptor mechanism whilst a more vague plateau is shown between 525 and 562 nm (Fig 5) Fig 6 demonstrates varying expressivity of the blue receptor with variations in localization of the stimulus and luminous intensity of the yellow background All curves have a maximum at 441 nm except curve A which indicates the sensitivity for the smallest object by central registration It is observed that the second plateau between 525 and 562 nm which shows the influence of the green receptor decreases with the greater luminous intensity in the background and can not be traced at all at the peripheral position 10 nasally (curve G)

Comments

In the conditioning lights only certain regions of the spectrum are illuminated while other regions are left in obscurity It is the darkened spectral region which chiefly determines the selectivity i e which one of the receptor mechanisms will be the dominant Therefore great importance has been attached to bring the eye in a stable condition of adaptation The purity of the sensitivity curves i e to which degree they represent the sensitivity of only one type of receptor is also dependant upon the luminous intensity of the bleaching light. The luminous intensities used here are rather low especially when compared with the very high intensities used by Wald (1964 1967) Stiles (1939 1964) used high as well as low luminous intensities and Marré (1969) comparatively low intensities Instead of the Maxwellian view presentation which has been used by all the authors mentioned the conditioning field in this set up has been the reflecting light from a diffuse white surface constituted by the perimeter sphere An adjustment of the background illumination to make it appropriate to the object light has been necessary In this connection the comparatively high transmission of the interference filters has proved to advantage The object size IV (16 mm) is specially suited for the peripheral registrations Furthermore with this large object the influence of a possible out of focus image upon the threshold value is of little importance (Sloan 1961)

Though the curves presented here can be said to have only moderate degrees of purity they are still dominated by the particular cone receptors which it has been the intention to bring forth Therefore in normal persons the sensitivity curves obtained by chromatic adaptation by this method are sufficiently distinct to separate and identify the individual cone mechanisms

With the smallest object size (1/16 mm²) comprising about 4 of visual angle there was a normal response from the red and green receptor types (Figs 3 & 4) whilst no response was observed from a blue sensitive cone type when the patient fixated centrally (Fig 6) However with the very same object at the position 2 paracentrally there was a clear response from the blue cone type Demonstrated here is the foveal blue blindness Wald (1967) ascribed this failing response in the fixation area of the fovea to lack of functional blue sensitive cones Hurvich (19 0) on the other hand supposed that the macula pigment had a maximal absorption in the very centre of the fovea and thus could explain the observed blue blindness in the fixation area

Likewise with the large object IV a relatively weakened sensitivity was found centrally for the blue receptor by static perimetry (Fig 9) With the chromatic adaptation technique each of the cone mechanisms reveal their characteristic perimeter profiles. Even though several factors may influence the course of the

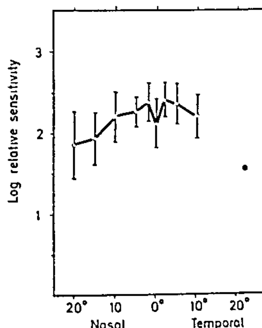


Fig 9

Static perimeter curve in yellow background with a blue violet object light (λ_{max} = 441 nm) The curve shows the average values of 5 normal individuals The variation (± 1 s d) is indicated by vertical lines

Figs 7 8 & 9 show the peripheral state of sensitivity as indicated by the static perimetry profiles within 20 of excentricity during adaptation to blue purple and yellow lights The perimeter curve shown in Fig 7 is dominated by the red cone mechanism There is an initial rapid decline in sensitivity near the fixation point after which the sensitivity loss is more gradual towards the periphery In Fig 8 where the sensitivity is dominated by the green cone mechanism it is evident that the curve is falling less steeply towards the periphery The characteristic feature of the curve shown in Fig 9 where the blue receptor mechanism is predominant is the marked decrease in the central sensitivity and instead a maximum sensitivity is found paracentrally at 2 from where the curve falls slowly towards the periphery With all three curves of static perimetry large individual variations are found at the 20 temporal position evidently due to the individual influence of the blind spot which is in accordance with the experience from ordinary static perimetry In one of the subjects no response was obtained at 20° therefore the value of 22 was indicated

Comments

In the conditioning lights only certain regions of the spectrum are illuminated while other regions are left in obscurity It is the darkened spectral region which chiefly determines the selectivity i e. which one of the receptor mechanisms will be the dominant Therefore great importance has been attached to bring the eye in a stable condition of adaptation The purity of the sensitivity curves i e to which degree they represent the sensitivity of only one type of receptor is also *dependant upon* the luminous intensity of the bleaching light The luminous intensities used here are rather low especially when compared with the very high intensities used by Wald (1964 1967) Stiles (1949 1964) used high as well as low luminous intensities and Marré (1969) comparatively low intensities Instead of the Maxwellian view presentation which has been used by all the authors mentioned the conditioning field in this set up has been the reflecting light from a diffuse white surface constituted by the perimeter sphere An adjustment of the background illumination to make it appropriate to the object light has been necessary In this connection the comparatively high transmission of the interference filters has proved to advantage The object size IV (16 mm²) is specially suited for the peripheral registrations Furthermore with this large object the influence of a possible out of focus image upon the threshold value is of little importance (Sloan 1961)

Though the curves presented here can be said to have only moderate degrees of purity they are still dominated by the particular cone receptors which it has been the intention to bring forth Therefore in normal persons the sensitivity curves obtained by chromatic adaptation by this method are sufficiently distinct to separate and identify the individual cone mechanisms

With the smallest object size (1/16 mm) comprising about 4 of visual angle there was a normal response from the red and green receptor types (Figs 3 & 4) whilst no response was observed from a blue sensitive cone type when the patient fixated centrally (Fig 6) However with the very same object at the position 2 paracentrally there was a clear response from the blue cone type Demonstrated here is the foveal blue blindness Wald (1967) ascribed this *failing response in the fixation area* of the fovea to lack of functional blue sensitive cones Hurvich (19 0) on the *other hand supposed* that the macula pigment had a *maximal* absorption in the very centre of the fovea and thus could *explain the observed* blue blindness in the fixation area

Likewise with the large object IV a relatively weakened sensitivity was found *centrally* for the blue receptor by static perimetry (Fig 9) With *the chromatic adaptation* technique each of the cone mechanisms reveal their characteristic *perimeter profiles* Even though several factors may influence the course of the

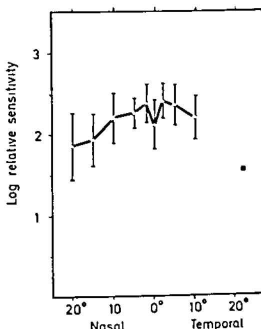


Fig 9

Static perimeter curve in yellow background with a blue violet object light (λ_{max} = 441 nm) The curve shows the average values of 5 normal individuals The variation (± 1 s d) is indicated by vertical lines

Figs 7 8 & 9 show the peripheral state of sensitivity as indicated by the static perimetry profiles within 20 of excentricity during adaptation to blue purple and yellow lights The perimeter curve shown in Fig 7 is dominated by the red cone mechanism There is an initial rapid decline in sensitivity near the fixation point after which the sensitivity loss is more gradual towards the periphery In Fig 8 where the sensitivity is dominated by the green cone mechanism it is evident that the curve is falling less steeply towards the periphery The characteristic feature of the curve shown in Fig 9 where the blue receptor mechanism is predominant is the marked decrease in the central sensitivity and instead a maximum sensitivity is found paracentrally at 2 from where the curve falls slowly towards the periphery With all three curves of static perimetry large individual variations are found at the 20 temporal position evidently due to the individual influence of the blind spot which is in accordance with the experience from ordinary static perimetry In one of the subjects no response was obtained at 20° therefore the value of 22 was indicated

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COLOUR FLUORESCEIN ANGIOGRAPHY OF THE FUNDUS

BY

VIGGO A JENSEN and WERNER OLSEN

Highly efficient interference filters the design and manufacture of which were controlled by computer programmes are described These filters may be used for a variety of purposes including immunofluorescence microscopy and retinal fluorescein angiography The filters are characterized by high transmission great selectivity and a steep front of the transmission curve Their use in the colour fluorescein angiography of the fundus is reported below

Key words interference filter – fluorescein angiography – colour fluorescein angiography

In order to take advantage of the greater amount of information provided by colour fluorescein angiography of the fundus as compared with black and white exposures several attempts have been made to devise special filters and combinations of filters which are capable of reproducing both the red and yellowish green colour during the passage of fluorescein containing blood through the retinal and choroidal vessels In their splendid atlas Shikano & Shimizu (1968) show examples of colour photographs taken by means of gelatin filters Mitsui

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static perimeter curves the results may justify the assumption that the three types of cones have different patterns of distribution in the retina

Acknowledgment

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wavelengths is *inserted* in the light path between the light source and the fluorescent object i e the so called primary beam It is therefore called the primary filter or exciting filter while the filter which blocks reflected excitation light if any in the light path used for observation i e the secondary beam is called the secondary filter or barrier filter The primary filter *is a short* (wave)pass filter while the secondary filter is a long (wave)pass filter The two filters cannot have any spectral transmission region in common since such a leakage would dim or drown the fluorescence. However it is possible to use filter systems in which the primary and secondary filters have a limited transmission region in common so that part of the light source is allowed to pass through both filters In such cases the exciting light is an entirely different colour from that of the fluorescent light It is on such filters that colour fluorescein angiography is based and the results of their use in clinical practice are presented in this paper *An intentional leakage in the filter system may thus result in* additive illumination of a suitable colour and it is possible to select a contrasting colour which decisively adds to the information provided by the fluorescein angiogram (Fig 2)

Primary filters

In the early phase of the development of the fluorescence technique ultraviolet light was always used for excitation In the use of gas discharge lamps of the *mercury vapour* type the only purpose of the primary filter was to block all visible light Subsequently it was realized that a better fluorescense could be *obtained by use of the specific wave lengths* which resulted in maximum sensitivity and maximum emission for the dye concerned (fluorochrome) Fluorescein reacted more intensely with blue filters and blue light gave much less autofluorescence i e fluorescence from unstained tissue whose proteins often reacted intensely to ultraviolet light

However the effect of blue filters was not optimal They either transmitted too little energy at the optimum wavelengths or they did not effectively cut off the forbidden wavelengths they were not sufficiently steep in the course of the transmission curve between the excitation and fluorescence ranges It is simply impossible to produce coloured glass with steep short pass transmission fronts whereas long pass glass filters with a well defined front anywhere in the spectrum can easily be made The latter are therefore still used as cheap and efficient secondary filters in modern fluorescence technique. Accordingly it was necessary to solve the problems involved in the development of a useful primary filter in a different way and in the search for a solution there was every reason to focus attention on the new discipline, thin film optics which is

Matsubara & Kanagawa (1968) and Matsui (1969) also used exciting gelatin filters Hendrickson Oniki & Elliot (1970) employed Kodak Wratten filters both as exciting and barrier filters The primary filter used by Blodi (1972) was a Leitz filter designed for fluorescence microscopy while his secondary filter was a Kodak Wratten No 15

Fluorescence technique

The use of fluorescein dyes for the observation of the blood flow through the ocular fundus is based on the utilization of the highly specific properties of these dyes When they are illuminated with light of a suitable wavelength they absorb part of the incident light energy and this energy is immediately emitted as light which in various ways differs from that of the illuminating source First it is scattered to all sides just as if the dye was an independent light source and secondly the emitted light is of a different colour It may be said that the fluorescein dyes transform the light from one wavelength to another and that the transformed light always has a wavelength which is longer than that of the exciting light This rule for the spectral shift is called Stokes Law These phenomena offer very favourable conditions for observing the dyes against a background which does not itself fluoresce Thus if the exciting light is from a limited wave range – and here we may choose that part of the spectrum which most efficiently is transformed by the dye – it is possible to observe the fluorescence on a dark background and the fluorescence is localized only to the dye

This can be achieved by two spectral limitations (1) by choosing wavelengths for the exciting light which are all shorter than the waves of the fluorescent light i e those we want to observe and (2) by excluding the exciting light from the observation The latter limitation might appear superfluous since the exciting and fluorescent lights are of different colours but it should be borne in mind that the difference in the colours is only small because the wavelength of the most efficient exciting light is very close to that of the fluorescence and also that the intensity of the fluorescence is many times weaker than that part of the exciting light which is reflected Thus an upper limit in the spectrum is set for the excitation at those wavelengths from which the fluorescence is emitted and the observation is restricted to the spectral window which is reserved for the fluorescence These spectral limitations are obtained by the use of optical filters

Fluorescence filters

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Fluorescence filters

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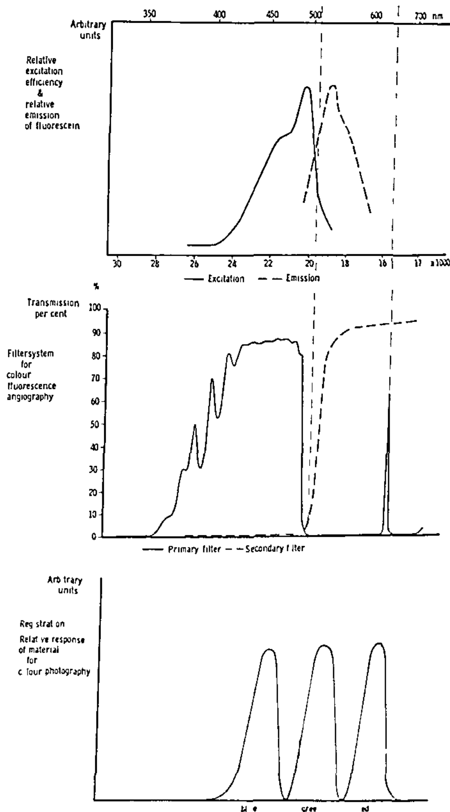
based on the fact that thin films of thicknesses of from one half to one quarter of a light wave can be built up layer upon layer so that they transmit or reflect well defined wave bands These filters are also called interference filters because their effect is based on the interference of light between the many thin layers – as distinct from the glass filters the effect of which is due entirely to absorption

Hodge & Clemett (1966) used an interference filter a band filter which transmits light in the range between 430 and 500 nm They reported that the transmission was 80% at 470 nm and did not exceed 1% between 480 and 500 nm This filter represents a substantial improvement as compared with the blue glass filters because the good transmission was obtained at more efficient wavelengths but the optimal excitation from 400 to almost 500 nm was not obtained This excitation could not be achieved until the Laboratory of Technical Optics in 1968 devised a proper short pass filter after being assigned the task by Danish pathologists to optimize the filter technique in order to fulfil the very strict requirements made on illumination in immunofluorescence microscopy The laboratory which employs large computers in the design of optical systems had at that time developed computer programmes which simulated the filtration of light by interference filters and could correct their spectral transmission to a high degree of perfection These programmes produced transmission curves in the course of a few minutes and the design of a primary filter could be managed within a reasonable period of time (Rygaard & Olsen 1969) During the next few years we were engaged in the development of these complex filters which are composed of up to 30 layers of varying thickness

A new method and a new design

However the reproducibility was poor and calculations showed that an accuracy better than 0 5% for each individual layer was required This low tolerance could be achieved only if the thickness of the layer was subjected to a strict control while it was being built up and for this control highly sensitive spectrophotometric measuring equipment was required At the same time it was necessary to elaborate a measuring technique that would give the greatest accuracy in the control of the thickness of each individual layer (This thickness is less than 0 0002 mm i e. a tolerance of 0.5% corresponds to 0 000001 mm)

These requirements were fulfilled by the development of programmes in our laboratory which could select the most sensitive wavelengths for the control



SPECTRAL CHARACTERISTICS OF FLUORESCENCE ANGIOGRAPHY IN COLOUR

Fig 1

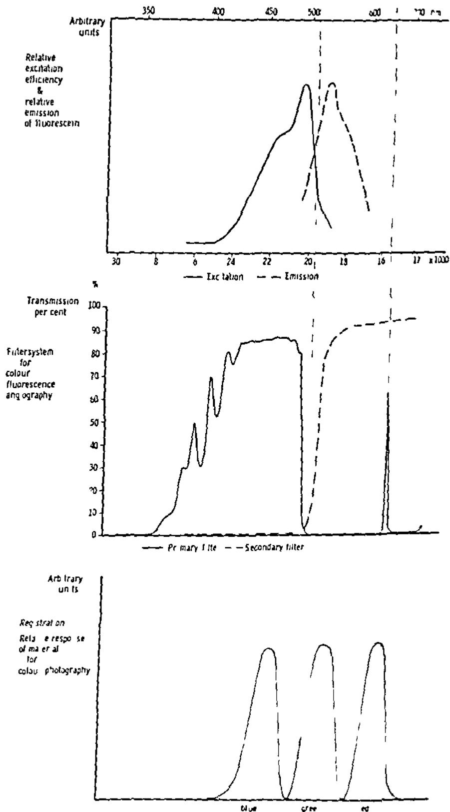
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SPECTRAL CHARACTERISTICS OF FL R RESCE E A CH AP IN C A R

Fig 1

of the thickness of each individual layer Thus a controlling scheme was calcu lated by the computer printed as a long list of strict directions for each of the many layers of the filter with information concerning the measuring wave lengths and the predicted transmission change during the production of the individual layer i e while the filter material condenses on the substrate in the high vacuum chamber

The development of this technique was completed in 1971 and the repro ducibility was extremely good A further computerized optimalization in 1972 led to what is theoretically obtainable, viz a primary filter of the short pass type the transmission of which is shown in Fig 1

The curve depicts a steep fall from more than 80 % transmission to 1 % over a range of less than 10 nm and an effective blocking with 0 01 % in the fluo rescence range (Rygaard & Olsen 1971)

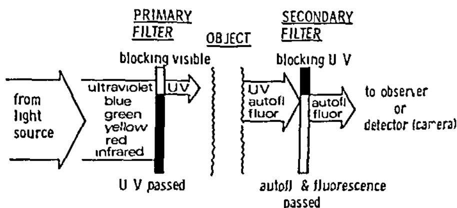
These filters are now used by immunologists and pathologists in many coun tries and we use it here in the Zeiss retinal camera in which the high excitation and good blocking of false light give a contrast which makes it possible to introduce a background colour This is shown as a very narrow band on the transmission curve at approx 660 nm The precondition for employing a con trasting colour for background illumination is obviously that two colours be used for detection i e the recording must be done by a colour sensitive system such as colour television or a colour film

Colour fluorescein angiography

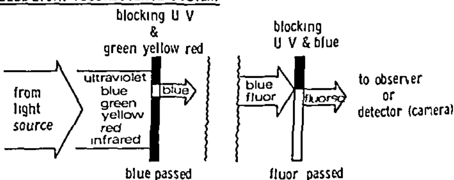
Angiography with fluorescein and contrast colour illumination in red makes it possible to observe and record a larger amount of information than by the conventional method employing blue excitation light The colour angiogram is much more differentiated because the two colour bands – the green fluorescence and the narrow red spectral band – result in all shades between green yellow orange and red according to the proportion between the red and green stimuli acting on the green and red sensibilization bands of the colour film or on the observer s green and red receptors

This opens up the possibility of observing a number of details and changes in the course of the blood flow in the retinal vessels which cannot be recorded in black and white angiography This differentiation in the colours occurs in seve ral fundamentally different ways The red light which passes through both the primary and secondary filters is scattered and is reflected according to the transparency and to the absorbing and reflecting properties of the fundus In the area where the fluorescence is built up by the passage of injected dye the addi tive composition with the red image gives rise to dynamic changes in a specific

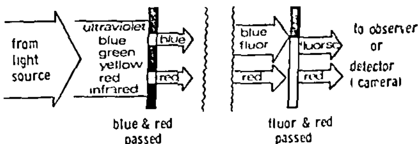
ULTRAVIOLET FLUORESCENCE SYSTEM



BLUE LIGHT FLUORESCENCE SYSTEM



COLOUR FLUORESCENCE SYSTEM



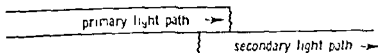


Fig 2

superficial vascular plexus of the disc and the cilioretinal artery if present. The fluorescence of the choriocapillaris imparts to the fundus a diffuse intense yellow colour which rapidly changes into the characteristic fine background mottling and at the end of the venous phase assumes an orange hue Corresponding to the dark spot seen in the macula on black and white films the posterior pole remains reddish and therefore provides a good contrast to the arterioles venules and the arcades of the capillary network around the avascular macular area where the red colour is most pronounced (Fig 7) The fluorescence of the retinal vessels follows the well known pattern The colours of the arteries and veins differ the latter being faintly orange which makes it possible to distinguish between the two types of vessels when they appear in films comprising only the periphery of the fundus In elderly patients a sparse greenish colour appears just outside the temporal disc margin in the arterial phase a little later changing into a temporal peripapillary halo

Occasionally the disc margin is – either wholly or in part – outlined by a bright yellow contour Atrophic papillary tissue white exudates and cicatricially exposed scleral tissue e g in toxoplasmosis assume a reddish tinge owing to the red band of the interference filter but autofluorescence does not occur If the choroid fluorescence is concealed by haemorrhages these retain their red colour Vessels in darkly pigmented fundi or running across a benign melanoma assume a more greenish tinge In the presence of malignant pigmented melanomata the exudation is more intensely green than in non pigmented tumours (Fig 5) Exudates from superficial vessels are even more intensely green

In the presence of papilloedema the first traces of fluorescein are seen in the fairly dense superficial vascular network – including the cilioretinal artery if present – of the temporal half of the disc Early in the arterial phase a circumpapillary cloudy greenish zone develops rapidly spreading across the papillary tissue and persisting for a long time after the fluorescence of the retinal vessels and the choroid has faded away (Fig 4)

In the incipient stage of recurrent choroititis fluorescein angiography immediately reveals the area from which exudation occurs (Fig 6)

A fluorescein angiogram showing capillary changes (Fig 7) has previously been published (Ehlers & Jensen 1973)

The foetal character of vascular malformations is manifested by momentary complete fluorescence even before the first weak traces of laminar flow through the veins occurs (Fig 8)

When, in spite of the best possible technique, the angiograms do not come up to expectations as regards intensity tone and contrast of the colours this may be due to individual biochemical variations in such factors as the binding of the

way disclosing a number of details of the vascular network and of any anomalies The differentiation of the green component also occurs in several ways. As the amount of dye changes with time the fluorescence as well as the excitation will change because the red blood intensely absorbs blue light and thus affects the excitation The areas which still contain unstained blood may then cover the areas in which fluorescein containing blood is present in the vessels or tissue and suppress their fluorescence Heavily pigmented areas will also reduce both the excitation and the fluorescence Individual features will thus affect both the intensity and the colour but clinical experience will easily compensate for these variations

The angiograms reproduced here were taken with a Zeiss retinal camera provided with a Robot Motor Recorder 36 ME The exciting interference filter is mounted in a holder which can be inserted into the light path of the xenon through the side wall of the camera The barrier filter mounted in a similar holder is placed in front of the Robot camera The generator is adjusted to 50 Wsec at time intervals of 1 2 sec To avoid excessive heating of the xenon lamp and resulting drop outs of flashes the lamp is cooled by the air current from a vacuum cleaner The colour film used is a Kodak Ektachrome High Speed (ASA 160 DIN 23) To facilitate the identification of the details of the fundus each film consisting of a series of 36 frames is started with a couple of exposures before fluorescein is injected but it is then necessary to temporarily replace the barrier filter by a 50 % neutral filter even if the generator is simultaneously adjusted to the lowest power out put in order to avoid over exposure of the film Three ml of a 20 % fluorescein solution is then injected into a cubital vein the pupil having formerly been dilated with 0 5 % Cyclogyl® (cyclopental hydrochloride)

The colour fluorescein angiograms primarily reveal details of the circulatory dynamics capillary permeability and vascular structures

The fluorescence may – not only in an entire series of exposures but also in the individual exposure – be more or less distinct varying in shade from yellow through green to orange according to the time localization and tissue structure These variations are of crucial importance in the differentiation between various forms of disc prominence such as pseudoneuritis drusen of the disc and papilloedema The angiograms reproduced in this paper are cut outs of a series of exposures and accordingly cannot convey the vivid impression of the blood circulation imparted by the successive viewing of the individual exposures of the series

Before the fluorescein has reached the eye the exposures are blurred very red and only a faint outline of the optic disc can be made out Just before the onset of choroid fluorescence the first traces of fluorescence can be seen in the

fluorescein to the plasma proteins or the haemoglobin level which may give rise to a shift in intensity of the absorbed and emitted light

It would probably be an improvement on the method if – instead of one exciting filter – three filters with slightly different characteristics were mounted consecutively on the filter selector wheel and coupled to the feed mechanism of the camera so that every third exposure was made with the same filter

The set of three filters should be composed of one filter represented by the curve in Fig 1 a second with the same excitation band but with the red contrast band suppressed to approx half transmission and a third filter with a still more reduced transmission in the red band Such filters can be produced without difficulty by means of the technique described in this paper

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Fig 3 Vitelliform macular retinopathy – girl aged 12 years



Fig 4 Papilloedema – man aged 3[illegible] years

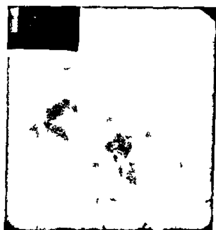


Fig 5 Metastasis from a mammary carcinoma – woman aged [illegible] years

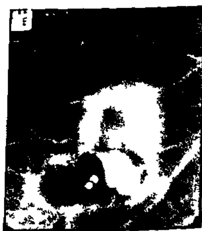


Fig 6 Recurrent choroiditis – woman aged 2[illegible] years

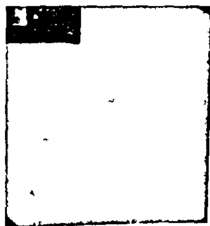


Fig 7 Hereditary haemorrhagic telangiectasis – woman aged 40 years



Fig 8 Vascular malformation (arteriovenous communication) – woman aged [illegible]0 years

In a previous paper I evidenced that the plaque is a further development of a *preplaque* (Norn 1973) A preplaque is a greyish band in front of a muscle insertion It is disclosed by illumination with a pencil lamp on gross examination

The *true plaque* on the other hand is only visible in the slit lamp best in indirect light By lighting to the left of the plaque its right border becomes plainly visible while a light beam to the right of the plaque discloses its left border

The incidence of scleral plaques was found to rise with increasing age from 3 % among the patients aged about 60 to 10 % among those aged about 70 and even to 25 % among the still older patients

The object of the present study was to control the development of scleral plaques by following up the patients from 6 to 12 months after the primary examination

To my knowledge no such follow up of scleral plaques has been undertaken previously with a view to studying their developmental pattern

Material

Within a two year period from October 1971 to October 1973 I saw 65 patients with scleral plaques in my ophthalmological practice (Vanløse)

These patients were summoned for follow up examination in groups every 6 months The individual subjects were thus examined between 6 and 12 months after the primary examination i e with an observation period of an average of about 9 months

Of these 65 subjects 47 (or 72 %) appeared for the follow up while 18 failed to appear for different reasons (unknown address illness feebleness and in two or three cases death)

Method

On the primary examination a sketch was drawn of the plaque and the following three plaque dimensions were measured with measuring ocular in

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SCLERAL PLAQUES

II Follow Up Cause

BY

M S NORN

A follow up (measurement by measuring ocular in the Haag Streit slit lamp) of 90 scleral plaques in 47 patients from 6 to 12 months after the primary examination showed the plaques to have increased in size on an average 40 % in height and 84 % in breadth growing more away from than towards the limbus corneae
The previously advanced theories concerning their cause desiccation poor blood supply and mechanical muscular action are discussed
The first theory is refutable by the growth direction of the plaque The third one is borne out by the growth direction of the plaque its site in front of the muscle insertion and by the plaque being found most frequently in front of the medial horizontal eye muscles

Key words sclera – scleral plaques – follow up – theories origin

A *scleral plaque* is a well defined superficial transparent scleral area covering a subjacent normal white non pellucid sclera The underlying uvea gives the plaque a greyish colour The plaque is situated in front of the insertion of an eye muscle

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The incidence of scleral plaques was found to rise with increasing age, from 3% among the patients aged about 60, to 10% among those aged about 70, and even to 25% among the still older patients.

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Of these 65 subjects 47 (or 72%) appeared for the follow up, while 18 failed to appear for different reasons (unknown address, illness, feebleness and in two or three cases death).

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On the primary examination a *sketch* was drawn of the plaque and the following three plaque *dimensions* were measured with measuring ocular in

the slit lamp 1) height 2) breadth and 3) distance from the anterior border of the plaque to the limbus corneae The measurement was made to the nearest 0 1 mm of the scale

At the follow up the shape of the plaque was drawn again and the same dimensions were measured Neither the original sketch nor the original measures were at my disposal at the follow up The follow up constituted in other words a kind of blank analysis The measurements were made in the Haag Streit slit lamp 900 in direct and indirect lighting in a half lit room

Result

The primary examinations had disclosed 90 scleral plaques in the series of 47 patients The follow up examinations showed not only all the original 90 plaques but in addition four new ones

The four new plaques occurred in four subjects who already had one or more plaques Two of the new plaques were circular (1 0 × 1 0 mm and 1 2 × 1 2 mm) while two were oval (1 5 × 0 7 mm and 2 8 × 1 0 mm)

The follow up showed that the great majority of plaques had increased in size during the observation period Of the original 90 plaques 66 had grown in height 17 had remained unchanged and 7 decreased The reduced plaque measures were however within the range of measuring errors The height therefore cannot be claimed to have decreased significantly

Table I

Alterations of scleral plaque dimensions at follow up 6 to 12 months after the primary measurement A total of 90 plaques Limbus is the distance from the anterior border of the plaque to the limbus corneae

	Number of plaques			Average alteration in per cent
	Increased	Unchanged	Decreased	
Vertical	66	17	7	39
Horizontal	61	20	9	34 3
Limbus	7	47	36	– 6 6

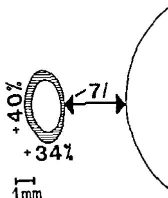


Fig 1

Average growth of scleral plaques The depicted plaque is vertically oval (2 2 × 1 47 mm) with a limbus distance of 3 96 mm The height increases on an average by 40 % and the breadth by 34 % *while the distance to the limbus corneae decreases by 7 %* in the course of about 9 months

The growth on the other hand was significant in the great majority of cases For the whole series collectively a linear rise was seen of the vertical plaque height on an of average 39 7 % compared with the initial measure the increase (plus or minus) having been estimated in each individual case.

The horizontal measure had likewise increased during the observation period though somewhat less so than the vertical (34 3 % see Table I)

Fig 1 illustrates the average result The original plaque was vertically oval It increased in size growing a little more in the vertical than in the horizontal direction

The distance between the limbus corneae and the nearest border of the plaque decreased in most cases (Table I) The linear distance decreased on an average of 6 6 % in the total series due to the plaque increasing in breadth thus coming nearer to the limbus (Fig 1)

If the plaque when increasing in breadth grew as much towards the limbus as towards the equator of the eyeball we should expect the limbus – plaque distance to decrease about 17 % (half of the total increase in breadth of 34 3 %) The figures suggest growth in both directions though mainly towards the equator and thus towards muscular attachment (Fig 1)

The growth in height and breadth depends on the size of the plaque. For the smallest plaques (≦ 1 0 mm) the average growth in height amounted to

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Table 1

Alterations of scleral plaque dimensions at follow up [illegible] months after the primary measurement A total of 90 plaques Limbus is the distance from the anterior border of the plaque to the limbus corneae

	Number of plaques			Average alteration in per cent
	Increased	Unchanged	Decreased	
Vertical	66	1		37
Horizontal	61	20	[illegible]	34 3
Limbus	[illegible]	4	80	6 6

circular plaques grow to become vertically oval Fig 2 I is a horizontally oval plaque which gradually attained a vertically oval shape owing to growth mainly in the vertical direction J-L *are examples of more irregular vertically* oval plaques M shows a plaque with *tongue shaped backward growth* The muscular attachment is just visible The plaque proceeds 0 5 mm under this The case is unique being the only one of my series in which the scleral plaque has extended so far under the muscular attachment

Conclusion

The follow up gave the result that scleral plaques grow on an average of 40% in height and 34% in breadth in the course of about 9 months Small plaques are most often circular The growth mainly in the vertical direction accounts for the vertically oval shape of most of the fairly large plaques (Norn 1974)

The growth in breadth proceeds mainly backwards in the equatorial direction but also to a smaller extent towards the limbus corneae

Small plaques grow the fastest older larger plaques at a slower rate No complications have been demonstrated such as scleralectasia staphyloma ulceration and perforation.

Discussion

Regarding the cause of scleral plaques Pillat as early as 1933 suggested the following three possibilities

I Desiccation

II *Poor blood supply*

III Mechanical muscular action

These possibilities *were discussed by* Cogan in 1959

The theory of desiccation has been supported in animal experiments by Fischer (19) and Casteiger (193) Fischer noticed grey patches on dislocated rabbit eyes and Casteiger provoked scleral plaques by instilling glycerin on the exposed sclera of live rabbits. These phenomena were however reversible

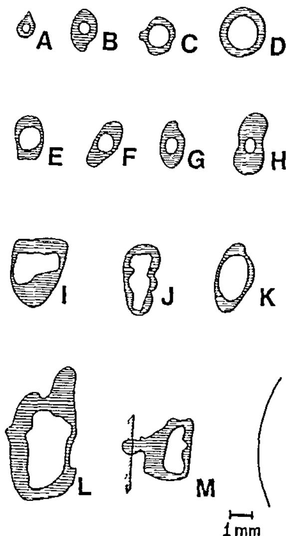


Fig 2

Different forms of scleral plaques and their growth within the observation period of 6 to 12 months Centrally is the original scleral plaque round which the outlines of the plaque have been drawn as they were seen at the follow up The hatched area represents the growth of the plaque Fig M also shows the limbus corneae (to the right) and the site of insertion of the rectus medialis

101 3% and that in breadth to 69 6% indicating that small early plaques grow the fastest The growth rate seems to decline at a later stage

Fig 2 (A–M) gives some examples of the growth of scleral plaques The central drawing represents the original plaque The surrounding hatched area shows its growth within the observation period A–H illustrate how originally

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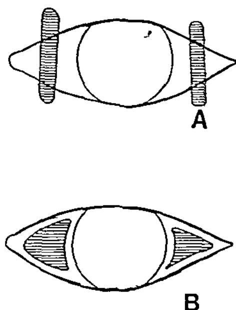


Fig 3

A The largest scleral plaque measured (12 × 6 mm) depicted in relation to the positions of the eyelids

B Desiccation triangles drawn on the sclera No scleral plaques of this appearance were found!

During prolonged operation the sclera may be seen to become transitorily translucent in spots due to desiccation However these spots are not well defined and the phenomenon is not identical with scleral plaques

If the theory of desiccation was correct we should expect a scleral plaque to grow in the exposed part of the sclera taking the shape of a triangle with its base along the limbus corneae and bounded by *the lid margins However* such growth has never been demonstrated (Fig 3 B)

A plaque grows mainly in the vertical direction *The largest plaque I have* seen was 12 mm high and 6 mm broad (Fig 3 A) This vertically oval plaque was partially covered by *the lids and was therefore not due to desiccation*

A *poor blood supply* to the sclera is a likely theory (*Boshoff 1942 Pur 1955* and Cogan et al 1959) The incidence of scleral plaques rises with increasing age Arteriosclerotic *changes minor emboli or thrombi may cause failing* blood supply to the scleral vessels especially to the branches of the anterior ciliary arteries passing *through the eye muscles*

We should expect scleral plaques to occur off the insertions of the different eye muscles I have only seen plaques in front of the horizontal eye muscles whereas neither on the first examination nor at the follow up were they seen in front of the vertical eye muscles

Scleral plaques in relation to vertical muscles have been described in only a few cases in the literature Pillat (1933) found in one patient thin plaques off the rectus inf o u and larger plaques off all four horizontal muscles Boshoff (1942) had a patient with plaques off both recti inf and also off three of the horizontal muscles Gasteiger (1937) observed a plaque running a curved course from the rectus lat to the rectus sup

All other cases have been described in relation to the horizontal muscles

Mechanical musclar action may involve wearing down of the scleral tissue which becomes transparent off the muscle insertion

The traction is exerted within the region just in front of the muscle insertion This accords well with the position and vertically oval shape of the plaque in front of the muscular attachment In one case only was a tongue shaped plaque prolongation seen to extend under the muscular attachment In all the other cases the plaque was situated anterior to the muscle

Scleral plaques are most frequently situated in front of the rectus medialis probably because this muscle is the one most commonly used (convergence)

Scleral plaques develop from preplaques which are vertically translucent regions likewise situated just before the insertion of the horizontal eye muscles

Scleral plaques are most likely due to an age determined localized wearing down of the sclera owing to continual traction caused by the adjoining eye muscle

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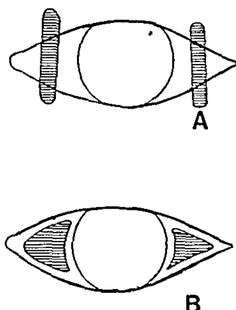


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THE ELECTRORETINOGRAM IN DIABETIC RETINOPATHY

A clinical study and a critical survey

BY

MAGNUS GJÖTTERBERG

The electroretinogram in 36 diabetics with different stages of retinopathy was studied A double flash technique enabling the registration of responses during scotopic mesopic and photopic conditions was used The a and b waves and the oscillatory potentials were assessed A tendency to hypernormal amplitudes of the b wave was noticed especially in the cases with background retinopathy Some cases with background retinopathy exhibited hypernormal oscillatory potentials but the only cases with constantly altered oscillatory potentials were those with a proliferative retinopathy in this group the amplitudes of the oscillatory potentials were always subnormal Alterations in the blood flow are suggested as the explanation of *the results obtained*

Key words diabetic retinopathy – electroretinography – oscillatory potentials.

The classical ERG is of limited value in assessing retinal damage caused by diabetes François & De Rouck (1954) and Karpe Kornerup & Wulfing (1958)

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found a reduced amplitude of the scotopic b wave only in cases with an advanced proliferative retinopathy Since Younemura Tsuzuki & Aoki (1962) described that the oscillatory potentials (o p) could be selectively reduced in amplitude already in slight diabetic retinopathy numerous studies on this subject have been published It has been difficult to correlate the stage of retinopathy with the reduction of amplitude of these potentials many studies are made on small case materials and the o p are often only roughly estimated as normal subnormal or extinguished sometimes on obscure grounds

In recent years Simonsen (1968) Brunette & Desrochers (1970) Tassy Jayle & Gastaut Maysou (1971) and Galloway Wells & Barber (1972) have presented studies of a larger number of cases Common to these investigations is that the correlation between the stage of retinopathy and the reduction of the amplitude of the o p is uncertain especially in the earlier stages Diabetics without any visible retinopathy may have hypernormal (Simonsen Brunette) as well as extinguished o p (Brunette Tassy) In background retinopathy a similar wide range of values is reported When proliferative retinopathy is established the o p generally are reduced in all case materials published It is somewhat difficult to compare the observations made in these investigations since different systems are used for grading the retinopathy The statistical procedures performed are also of varying quality another problem is the different ways used to elicit record and measure the o p

The common mode of eliciting o p for clinical assessment has been to strive for maximal amplitudes. It cannot be said that this is the most delicate way to detect disturbances in the behaviour of the o p Tassy et al (1971) considered the o p to be reduced more under dark adaptation than under light adaptation (in diabetic retinopathy) Ghem Moller & Kietzmann (1971) reported that by using a lower stimulus intensity they registered more significant differences between diabetics and normals

According to Simonsen (1969) reduced o p are a bad prognostic sign this increases the need of further investigations about the correlation between the development of diabetic retinopathy and the changes of the o p In a previous work Algvere & Gjotterberg (1974) were able to correlate different stages of proliferative retinopathy assessed by fluorescein angiography with the reduction of the o p

The aim of the present investigation was to study the ERG including the a wave b wave and o p in different stages of diabetic retinopathy especially in the early stages In order to get information about the behaviour of the ERG under different conditions the double flash technique described by the author (Gjötterberg 1974) has been used With this technique photopic as well as scotopic responses can be obtained

Material and Methods

This series included 36 diabetics 15 women and 21 men Their ages ranged from 19 to 40 years the mean age being 31 years All patients developed diabetes before the age of 35 Sixteen patients developed diabetes before the age of 14 None of the patients had any grave complications of diabetes such as hypertension persisting albuminuria or evident disturbances of the peripheral circulation

All patients underwent an ophthalmological examination including the determination of refraction, the measurement of the intraocular pressure ophthalmoscopy and biomicroscopy If there was any difficulty in grading the retinopathy fluorescein angiography was performed (15 case) One eye was selected for electroretinography

The ERG including the o p was recorded in response to double flashes of strong light delivered from an xenon arc lamp The intervals ranged from 0 35 to 180 sec The signals were led off to a differential amplifier and displayed on a dual beam oscilloscope One sweep showed the a and b waves The other sweep being filtered displayed the o p Calibration was checked at each examination A pulse generator delivering a stable voltage of 200 μV was used The amplitudes of the different components were evaluated by caliper square measurements The error of measurement was estimated at $\pm$ 0 5 mm corresponding to $\pm$ 12 μV when measuring the a and b waves When measuring the o p $\pm$ 0 5 mm corresponds to $\pm$ 6 μV These errors have been considered small in comparison with the recorded amplitudes Measuring the photopic oscillations however the error of measurement cannot be totally ignored (For details of method and procedure see Gjotterberg 1974)

Results

According to the findings at the ophthalmological examination the patients were graded into three groups

A No evidence of retinopathy

B Background retinopathy (retinal angiopathy without any signs of neovascularisation i e simple retinopathy)

C Proliferative retinopathy (all stages of neovascularisation)

Ten patients belonged to group A twenty to group B and six to group C The mean duration of diabetes (after the age of 14) was in group A 8 years B 14

found a reduced amplitude of the scotopic b wave only in cases with an advanced, proliferative retinopathy Since Younemura Tsuzuki & Aoki (1962) described that the oscillatory potentials (o p) could be selectively reduced in amplitude already in slight diabetic retinopathy numerous studies on this subject have been published It has been difficult to correlate the stage of retinopathy with the reduction of amplitude of these potentials many studies are made on small case materials and the o p are often only roughly estimated as normal subnormal or extinguished sometimes on obscure grounds

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years and C 17 years This series did not include any patients with evident intravitreal fibrous proliferations or retinal detachment. The visual acuity in *general was good all patients in group* A had a visual acuity of 1 0 in group B one patient had a visual acuity of 0 7 the rest 1 0 In group C one patient had a visual acuity of 0 3 the rest 0 7 or better

Fig 1 shows the results of the ERG registration in four typical cases The recordings are selected to show scotopic response (D A) and mesopic (transition phase between photopic and scotopic conditions) responses at the 30 sec interval at the 1 sec interval photopic responses are obtained The recordings from four patients with different stages of retinopathy are shown

Case 1

35 year old woman Diabetes recently discovered Dilated retinal veins no other signs of retinopathy Hypernormal a wave at dark adaptation hypernormal o p at 30 and 1 sec intervals

Case 2

31 year *old man diabetes since the age of 8 Ophthalmoscopy and fluorescein* angiography disclosed numerous microaneurysms and small haemorrhages Solitary soft exudates No neovascularisation Hypernormal o p at 1 sec interval

Case 3

31 year old man diabetes since the age of 7 Numerous microaneurysms and small haemorrhages were observed but also small pathological intra retinal vessels as in early neovascularisation Hypernormal a waves at all three registrations presented Hypernormal b *wave at 30 sec interval Subnormal o p at* dark adaptation and at 30 sec interval

Case 4

23 year-old woman diabetes since the age of 4 Retinal neovascularisation with small tufts of preretinal vessels Hypernormal a wave at 30 and 1 sec intervals O p extinguished in all records

Fig 2 shows the recorded amplitudes of the a and b waves as well as the added amplitudes of the o p in all patients examined Some single registrations

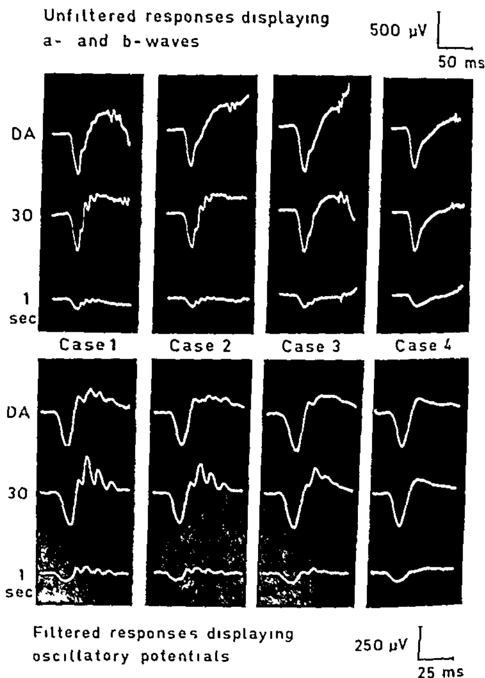


Fig 1

Examples of ERG recordings in 4 patients with diabetic retinopathy of different severity Recordings in dark adaptation (D A) and after the 30 and 1 sec intervals show scotopic, mesopic and photopic responses respectively For details of individual cases see text

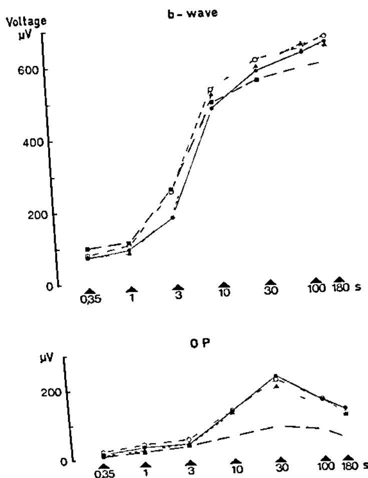


Fig 3

The mean values of the b waves and the o p in the three groups of diabetic retinopathy together w th the corresponding values in the normal material The tendency to hyper n rmality of the b wave is obv ous especially in the group with background retino pathy the difference is significant at the 3 and 10 sec intervals. The only group with subnormal values of the o p is the one with proliferative retinopathy For explanation of symbols see Fig 4

have been discarded because of unsatisfactory cooperation or technical mishaps The 95 % confidence limits for a normal material are indicated (see Gjotterberg 1974) For further explanation the reader is referred to the legend for Fig 2

As regards the a and b waves, the scotopic as well as the photopic responses

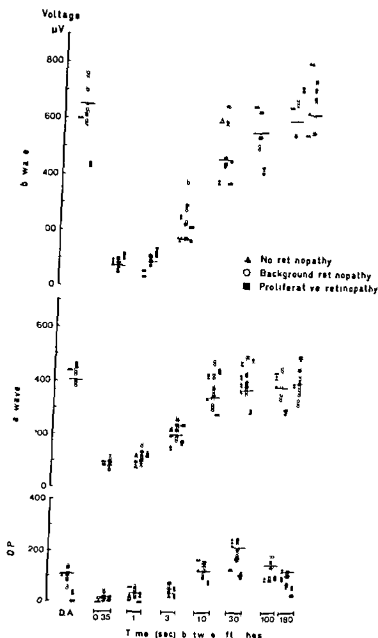


Fig 2

The recorded amplitudes of the a and b waves and the added amplitudes of the oscillatory potentials (o p) in all cases examined The mean and 95 % confidence limits (M ± 2 09 s) for a normal material are indicated by unbroken and dashed lines respectively ERG amplitudes in dark adaptation (D A) and after different intervals of stimulation as denoted by numerals (seconds)

The a and b waves exhibit a tendency to hypernormality rather than subnormality The registrations of the o p show the most decisive results after the 30 sec interval all cases with proliferative retinopathy exhibit subnormal values some cases with background retinopathy show hypernormal values

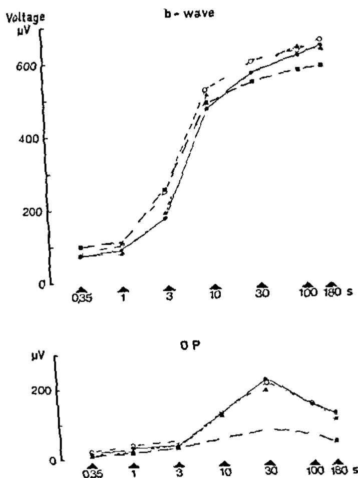


Fig 3

The mean values of the b waves and the o p in the three groups of diabetic retinopathy together with the corresponding values in the normal material The tendency to hyper normality of the b wave is obvious especially in the group with background retino pathy the d fference is significant at the 3 and 10 sec intervals The only group with subnormal values of the o p is the one with proliferative retinopathy For explanation of symbols see *Fig 4*

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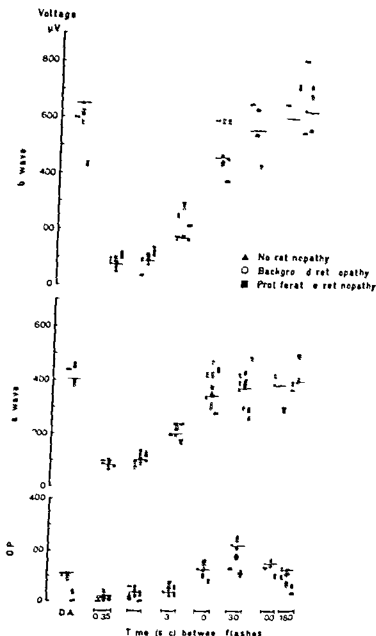


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Fig 3 shows the mean values of the b waves and the o p in the three groups together with the mean values recorded in the normal case material The mean values of the b waves in the group with background retinopathy are higher through the whole range of intervals from scotopic to photopic conditions The difference is statistically significant at the 3 and 10 sec intervals ($P < 1\%$ and $P < 5\%$ respectively two sample t test) The values of the group without detectible retinopathy follow the normal values closely The mean values of the amplitudes recorded in the cases with proliferative retinopathy exhibit a less steep curve starting from subnormal values under scotopic conditions and ending in hypernormal values under photopic conditions The difference is significant at the 3 sec interval ($P < 2\%$)

As regards the o p the only group which exhibits subnormal values is that with proliferative retinopathy The difference is significant during mesopic conditions (30 sec interval) but uncertain under photopic conditions

The mean amplitudes of the a waves in the three groups and the normals are compared in Fig 4 A slight tendency to hypernormal values is noticed but the difference is less pronounced than for the b waves

Discussion

The separate components of the ERG are differently influenced by disturbances in the retinal circulation Clamping the retinal circulation promptly reduces the b wave while the a wave is not reduced at all This has been demonstrated in monkeys (Brown & Watanabe 1962) In clinical investigations of humans with retinal vasculatory disease a corresponding difference in sensibility of the b wave and the a wave has been noticed However the b wave is not distinctly reduced until the circulation has seriously deteriorated (Rendahl 1958 Ponte 1968 Nilsson 1971 and others) The o p on the other hand are considered to be relatively more vulnerable than b wave to disturbances of the retinal circulation (Younemura Tsuzuki & Aoki 1966 Usami 1966 Kurachi Hirose & Younemura 1966 Algvere 1968 and others) The different sensibility of the three components can probably be explained by their separate cellular origin and the different vascular supply of these cells

In the present study the amplitudes of the a and b waves were not reduced in diabetics without any detectible retinopathy or with background retinopathy Neither did the cases with an early proliferative retinopathy exhibit any regular reduction of these potentials. Even the photopic b wave was normal This is in

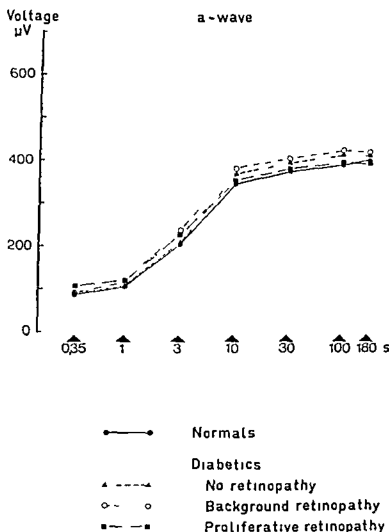


Fig 4
The mean values of the a waves exhibiting a slight tendency to hypernormality especially in the group with background retinopathy

only a few subnormal values were recorded this was valid for all three stages of retinopathy On the contrary there was an obvious tendency to hypernormal amplitudes This tendency was most pronounced at the 3 sec interval and for the patients with background or proliferative retinopathy

The values of the o p showed the most obvious changes at the 30 sec interval Three patients with background retinopathy exhibited hypernormal values All six cases with proliferative retinopathy showed subnormal values At more extreme scotopic or stronger photopic conditions the divergence from the normals was not so conspicuous

Fig 3 shows the mean values of the b waves and the o p in the three groups together with the mean values recorded in the normal case material The mean values of the b waves in the group with background retinopathy are higher through the whole range of intervals from scotopic to photopic conditions The difference is statistically significant at the 3 and 10 sec intervals ($P < 1\%$ and $P < 5\%$ respectively two sample t test) The values of the group without detectible retinopathy follow the normal values closely The mean values of the amplitudes recorded in the cases with proliferative retinopathy exhibit a less steep curve starting from subnormal values under scotopic conditions and ending in hypernormal values under photopic conditions The difference is significant at the 3 sec interval ($P < 2\%$)

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In the present study the amplitudes of the a and b waves were not reduced in diabetics without any detectible retinopathy or with background retinopathy Neither did the cases with an early proliferative retinopathy exhibit any regular reduction of these potentials Even the photopic b wave was normal This is in

good agreement with previous observations made by other authors (Jacobson 1961 Jacobson Hirose & Popkin 1967 and Tassy Jayle & Gastaut Maysou 1971)

The abnormality observed in the present study on the contrary, was a tendency to hypernormal amplitudes Gliem Moller & Kietzmann (1973) found by the use of a different double flash technique a reduction of the amplitude of the photopic b wave in cases without any visible retinopathy as well as in cases with different stages of diabetic retinopathy The comparison of these contradictory results is *complicated by the fact that Gliem et al used a different* method of eliciting the responses the interval between the stimulus flashes was constant and the time between the pairs of flashes was varied This makes the interpretation more complicated as does the fact that no examples of recordings are presented

More surprising is that in the present study the amplitudes of the o p were within normal limits in most diabetics Looked upon as groups there was no *significant difference between the recorded amplitudes in normals diabetics* without any detectible retinopathy or diabetics with background retinopathy Some individuals with slight retinopathy single microaneurysms and/or venous dilation showed hypernormal o p but otherwise most values obtained were within normal limits On the other hand all diabetics with proliferative retinopathy exhibited subnormal amplitudes of the o p These significant observations were made at the 30 sec interval i e in the present study the interval that elicit*ed the highest amplitudes*

Are these results in accordance with those presented by other authors? It is obvious that they differ from the observations made by some writers Kojima et al (1966) found a decrease or disappearance of o p in 64 % of diabetics without retinopathy Ohtsubo (1970) observed reduced o p in prediabetics These findings are not supported by the results of the present study

Brunette & Desrochers (1970) found subnormal or extinguished o p in about 25 % of patients with diabetes but without visible retinopathy However the statistical pretensions of subnormal were modest the confidence interval for normals was only about 65 % If a 95 % interval had been used the number of subnormals would have decreased considerably In proliferative retinopathy the o p were generally reduced It is interesting that Brunette & Desrochers found a natural decrease of *the amplitudes of the o p in higher ages over 50 years* of age This is not surprising since the b wave also decreases in the elderly (Karpe Rickenbach & Thomasson 1950 Peterson 1968)

Tassy Jayle & Gastaut Maysou (1971) examined a large number of diabetics by electroretinography Fluorescein angiography was not used to grade the retinopathy The authors studied the o p in dark and light adaptation They

found that the dark adapted o p were more vulnerable It is known that it is difficult to register distinct o p in dark adaptation even in normals (Algvere & Westbeck 1972) In a previous work (Gjotterberg 1974) the results of which create the normal material of this study the values of the o p recorded in dark adaptation showed a wide range the lower limit of the 95 % confidence interval was almost at zero This makes the a sessment of values obtained in dark adaptation difficult. In the present study the most significant observations were made at the 30 sec interval where maximal amplitudes were obtained

Nagata (1962) has shown that with a short stimulus time less than 25 msec the o p are overshadowed by the off effect (h wave) during photopic conditions Tassy et al used a short stimulus time 50 μs while in the present study a stimulus time of 25 msec was used This makes the comparison of the photopic o p difficult but nevertheless the author agrees with Tassy et al in claiming that the photopic oscillations are less vulnerable

Simonsen has made impressive studies of the o p in diabetics (1968 1969) He found a wide range of values in diabetics with background retinopathy from subnormal to hypernormal amplitudes In proliferative retinopathy the o p were reduced or extinguished Simonsen has also noticed that cases with reduced o p have a worse prognosis The level of blood sugar did not influence the amplitudes of the o p Simonsen graded the retinopathy into three classes no retinopathy background retinopathy and proliferative retinopathy Fluorescein angiography was not performed A decisive question is whether fluorescein angiography would not have changed the grading of some eyes If so perhaps the correlation between the stage of retinopathy and the reduction of the amplitudes of the o p would have been better

In the present study as well as in the works of Simonsen and Brunette & Desrochers some cases exhibited hypernormal o p Karpe Kornerup & Wulfing found some diabetics to show hypernormal scotopic b waves the explanation was supposed to be venous stasis In the present investigation two of the three cases who exhibited hypernormal o p showed venous dilatation at ophthalmoscopy and fluorescein angiography In Fig 3 it was shown that especially the group with background retinopathy exhibited hypernormal b wave amplitudes Kohner (19 1) has noticed that diabetics with no retinopathy or with background retinopathy have an increased retinal blood flow It is likely that such an increased blood flow would result in high ERG amplitudes if the non perfused and damaged areas are not too large When the increase of blood flow is no longer able to compensate for the spreading of badly perfused areas a successive decrease of the retinal mass response ERG will be the result and the o p are obviously the first to be affected The unsuccessful attempt of the retina to improve the perfusion by neovascularisation does not result in a better ERG

in a work by Algvere & Gjötterberg (1974) it is shown that with progression of proliferative retinopathy from the earliest signs of neovascularisation to established preretinal proliferations, there is a progressive deterioration of the op

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Augenklinik des Bereiches Medizin der Universitat Rostock – DDR
(Direktor OMR Prof Dr sc med G Pietruschka)

BEITRAG ZUM ULLRICH-FREMEREY DOHNA-ODER FRANÇOIS-SYNDROM*

VON

MANFRED TEUSCHER

Nach kurzer Literaturubersicht wird uber einen an der Augenklinik des Bereiches Medizin der Universitat Rostock beobachteten Fall von François Syndrom berichtet Der derzeitige Status wird an Hand von 4 Bildern dargestellt

Key words syndrome of Ullrich Fremerey Dohna François – malformations

Im Jahre 1953 beschreiben Ullrich und seine Mitarbeiterin Fremerey Dohna einen Symptomenkomplex von

Dyscephalie
Schadelnahtanomalien
Mikrogenie und Mikrostomie
Mikrophthalmus
Cataracta congenita
Hypotrichose in Form suturaler Alopezie oder Alopezia areat
Sklerotisch atrophische Hautveranderungen im Bereich des ventralen Kopfsegmentes
Proportionierter Zwergwuchs
und normale geistige Entwicklung (31)

* Auszugsweise vorgetragen auf der Tagung der Berliner Augenarztlichen Gesellschaft im November 1973



Abb 1
Darstellung des Gesichtes mit der typischen Physiognomie

Dieser Symptomenkomplex ging spater als Ullrich Fremerey Dohna Syndrom *in die Literatur* ein und wurde gleichbedeutend fur die Synonyma Dyscephal odermatophakie Dyskraniodysopie und Dyscephalie mit Cataracta congenita und Hypotrichose gebraucht Es muss jedoch festgestellt werden dass beide Autoren nicht als erste diese Symptomenkombination beschrieben denn bereits 1893 teilten Aubry (1) und 1911 Bergmeister (3) deragtige Falle mit wie aus der sehr umfangreichen und alle Falle erfassenden Arbeit von François aus dem Jahre 1957 sowie aus denen von 1958 und 1960 zu ersehen ist (11 12)

François selbst konnte zu den von ihm zusammengestellten 21 Fallen der Weltliteratur noch 3 weitere hinzufugen die er selbst beobachtet hatte (12 13 14)

Seit dem Jahre 1955 erscheint der oben beschriebene Symptomenkomplex auch unter der Bezeichung François Syndrom oder dyscephalic syndrome nach François in der Literatur (6 8 15 19 25 33 36)

Nach den grundlegenden Arbeiten von Ullrich Fremerey Dohna und François erschienen weitere Fallberichte sodass die zur Zeit bekannte Fallzahl von François Syndrom etwa 30 betragt (7 8 19 27 28 30 33 36)

Seit dem Jahre 1966 haben wir an der Augenklinik des Bereiches Medizin der

*Augenklinik des Bereiches Medizin der Universität Rostock – DDR
(Direktor: OMR Prof. Dr. sc. med. G. Pietruschka)*

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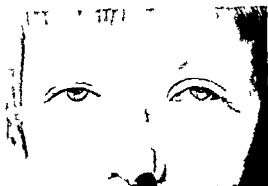


Abb 3
Darstellung der sklerotisch atrophischen Hautveranderungen im Bereich der Nase und der Wange

grossen zirkularen peripapillaren Herd feiner graulich pigmentierter Tupfelung Am linken Fundus befand sich die Papille in einem grossen deutlich begrenzten chorioretinitischen Herd der sich weit nach nasal erstreckte und zum Maculage biet hin in einer weisslich atrophische Narbe auslief

Mit einer Übungsbrille bds + 13 0 dpt erfolgte am 10 6 1966 die Entlassung



Abb 4
Darstellung der bei dem Kinde bestehenden Alopezia areata im Bereich des Hinter kopfes



Abb 2
Seitendarstellung des beschriebenen Falles (Kopf)

Universitat Rostock Gelegenheit ebenfalls ein Kind mit einem François Syn drom zu beobachten uber welches nun berichtet werden soll

Die erste Vorstellung des 1965 geborenen Kindes erfolgte im Alter von 1 1/4 Jahren wegen des Verdachtes auf einen fruhkindlichen Hirnschaden der von der Universitats Kinderklinik (Direktor Prof Dr sc med J Kulz) geaussert worden war Dabei fiel von seiten unseres Fachgebietes eine totale Linsentrub ung und ein Horizontalnystagmus auf Wegen der bestehenden Linsentrubung wurden zunachst Untersuchungen zum Ausschluss eines Diabetes einer Toxo plasmose einer Listeriose einer Tetanie sowie einer Myotonie vorgenommen Das Ergebnis war negativ Ausserdem fanden wir bei dem Kind eine Dyscepha lie eine Hypotrichose in Form einer Alopezia areata im Bereich des Hinter kopfes sowie sklerotisch atrophische Hautveranderungen die besonders deutlich im Bereich der Nase ausgepragt waren Desweiteren bestand eine Mikrognathie mit konsekutivem Sperrbiss und Zahlstellungsanomalien

Auf Grund der von uns erhobenen Augen und Allgemeinsymptomatik ordneten wir das Krankheitsbild als François Syndrom ein Am 23 5 66 fuhrten wir die Linsenablassung bds durch Nach komplikationslosem Heilverlauf erfolgte drei Wochen spater eine Narkoseuntersuchung des Augenhintergrun des Dabei stellte sich am rechten Fundus die Papille vital aber unscharf be grenzt dar, ein Gefasstrichter war nicht zu finden Die Papille lag in einem

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Anlasslich einer Narkoseuntersuchung 1970 fanden wir bds eine Papillen prominenz von + 4,0 dpt Die Papillen erschienen durch Einlagerung von glio sem Gewebe *insgesamt vergrossert und allseits unscharf begrenzt*

Die beschriebenen peripapillären Veranderungen bestanden weiter unver andert

Der Visus betrug zu diesem Zeitpunkt

R + 19 0 = 1/12 BT

L + 19 0 = 1/10 BT binokular 5/25 BT

Auf Grund des Papillenbefundes erfolgte eine stationare Untersuchung in der Abteilung fur Kinder Neuro Psychiatrie (Direktor Prof Dr sc med G Goll nitz) an der Universitats Nervenklinik Rostock wo ein intrakranieller raum fordernder Prozess mit Sicherheit ausgeschlossen werden konnte

Wir fassten deshalb den Papillenbefund als Pseudostauungspapille mit zir kumpapillärer Chorioidalatrophie unklarer Genese auf

Bei einer im Herbst 1973 durchgefuhrten Kontrolluntersuchung ergaben sich *hinsichtlich Visus Befund an Augenvorderabschnitten und am* Fundus keine neuen Gesichtspunkte

Diskussion

Der von uns beobachtete Fall zeigt alle typischen Merkmale eines François Syndrome

Das Verschwinden des Nystagmus nach erfolgreich durchgefuhrter Katarakt operation wie es von Torres Marty (30) beschrieben wurde konnten wir bei unserem Falle nicht finden

Durch Carones (8) wurden Netzhautdystrophien mit gekornter Pigmentation am hinteren Augenpol bei François Syndrom beobachtet Inwieweit die von uns beobachteten peripapillären Pigmentverschiebungen als ebensolche Erscheinung gewertet werden konnen ist nicht mit Sicherheit zu sagen

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BIONDI LIKE CELL INCLUSIONS IN THE HUMAN IRIS

BY

AMUND RINGVOLD

Characteristic circular ovoid and racket shaped cell inclusions (usually termed Biondi bodies) have previously been observed within the epithelium of the choroid plexus These bodies containing pigment lipids and fiber bundles appear *with increasing frequency from the fourth decade of life* and up to now they apparently have not been observed in any other tissue In the present communication similar inclusions have been demonstrated within stromal cells of the human iris The function of these bodies is by no means clear but it is pointed out that morphologically they bear strong resemblance to lysosomes

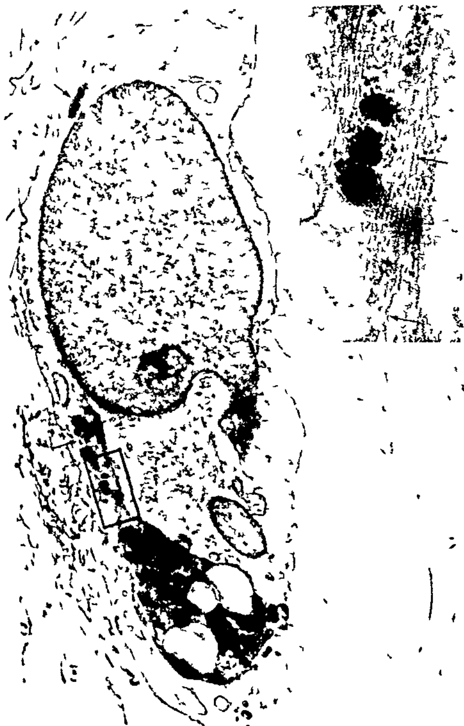
Key words Biondi bodies – iris – lysosomes – electron microscopy – cytology

Characteristic ring shaped cell inclusions have been demonstrated in the human choroid plexus by Biondi (1933) This observation has later been confirmed by several authors (Bargmann 1955 Bargmann & Katritsis 1966 Oksche & Vaupel von Harnack 1969 Dohrmann 19 0 Oksche & Kirschstein 19 ?) and it turned out that such bodies occur in older people only as also suggested by Biondi So far no specific function has been related to these elements which in some re

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ports are regarded as being degenerative in nature During a previous study some peculiar organelles were found in iris cells from normal adult human eyes Since these inclusions resemble the Biondi bodies of the choroid plexus and since Biondi like inclusions have apparently not been recognized beyond the brain the special inclusions of iris cells will be dealt with in the present paper

Material and Methods

Light microscopy

Light autopsy iris specimens (4 ♀ and 4 ♂) were used for this part of the study The patients were 86 82 82 79 74, 66 60 and 27 years old at death and so far as could be checked (from history and inspection) the eyes appeared normal Iris tissue was fixed in Susa or Bouin solutions dehydrated in alcohol embedded in celloidine paraffin and stained with hematoxylin eosin PAS or according to the procedure of v Gieson

Electron microscopy

Five biopsy iris specimens were available for this study These patients were by chance all women and they were 84 7? 74 69 and 63 years old at the time of operation One eye was enucleated because of intrabulbar melanoma at the posterior part of the bulbus whereas the other four specimens were removed during extraction of senile cataract Otherwise none of the five eyes showed signs of eye disease The iris specimens were immediately fixed for 1–2 hours in precooled 1 % OsO_4 buffered to pH 7 4 with phosphate buffer The material was dehydrated in graded acetone solutions and embedded in Araldite Sections were made with an LKB Ultrotome and stained with uranyl acetate followed by lead citrate Siemens Elmiskop 1 and 1A were used

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Fig 1

Electron micrograph of iris stroma cell with Biondi inclusion sending branches through great parts of the cytoplasm (arrows) × 13 500

Upper right inset higher magnification of the boxed area Doubled fibrils at arrows × 50 000



Fig 3

Electron micrograph showing Biondi inclusion with numerous offshoots containing [illegible] (arrows). Small and medium sized electron dense granules within the inclusion are seen. L lipid droplet. 60 000

Results

Most of the cells containing the inclusions described below were ovoid in shape and some of them had long cytoplasmic processes The nucleus was relatively large and the cytoplasm showed a pronounced Golgi apparatus and was rich in granular endoplasmic reticulum (Fig 1) Outside the inclusions concerned the cytoplasm contained very few melanosomes The real nature of these cells has not been definitely identified but they were not supposed to be melanocytes

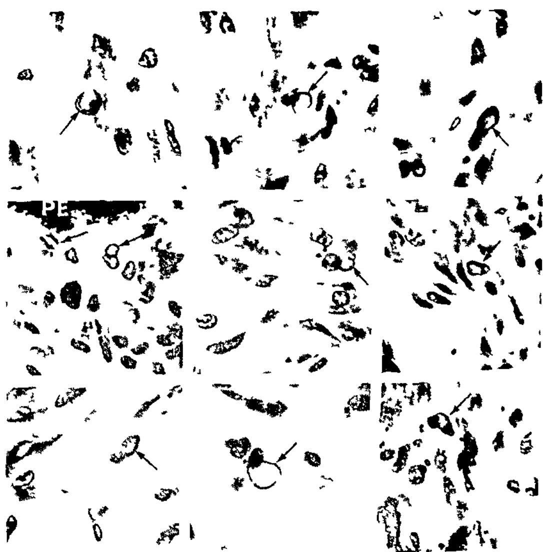


Fig 2
Light micrographs showing different forms of Biondi inclusions in iris stroma cells (arrows) PE – pigment epithelium Susa fixation HE stained × 400



Fig 3

El t n mi r gr ph h wing B ond inclusi n with numerous offshoots containing
t t l arrow Sm ll and med um s zed electron dense granules within the inclusion
are seen l l j d dr plet 60 000

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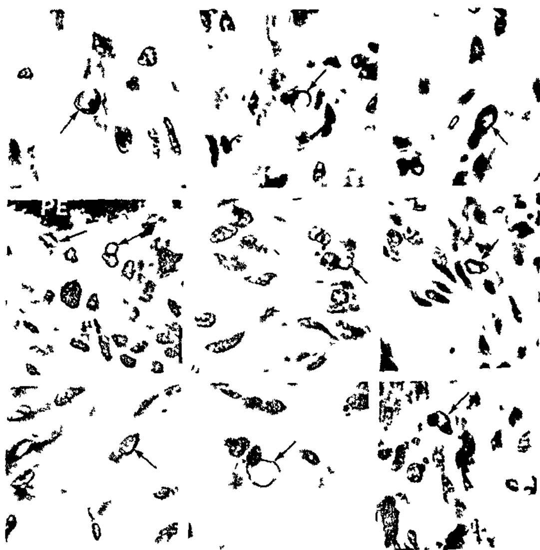


Fig 2

Light micrographs showing *different forms of Biondi inclusions in iris* stroma cells (arrows) PE – pigment epithelium Susa fixation HE stained × 700

shaped, rod-like or racket-like. In order to summarize the varieties of form some examples are given in Fig. 2.

The inclusions were most easily seen in tissue exposed to Susa fixation and stained with hematoxylin-eosin.



Fig.

Electron micrograph of inclusions lacking the characteristic offshoots at least at the level concerned. All components found in Biondi inclusions are present. × 13 500. Few doubled fibrils about 20 Å in diameter are shown in the inset, which is a higher magnification of the boxed area. × 50 000.

Light microscopy

The light microscopic appearance of the inclusions was rather varied The most striking ones were ring shaped (complete or incomplete with a marked enlargement somewhere along the circumference) Others were ovoid arched sickle



Fig 4

Electron micrograph of a Biondi inclusion distinctly limited by a triple layered membrane The two large dense bodies are supposed to be melanosomes L – lipid droplet × 60 000

haped rod like or racket like. In order to summarize the varieties of form ome examples are given in Fig 2

The inclusions were most easily seen in tissue exposed to Susa fixation and tained with hematoxylin eosin



Fig

Electron micrograph of inclusions lacking the characteristic offshoots at least at the level concerned All components found in Biondi inclusions are present × 13 800 Few doubled fibrils about 90 Å in diameter are shown in the inset which is a higher magnification of the boxed area × 50 000

Electron microscopy

The inclusions were composed of a large body with some thin offshoots radiating into the surrounding cytoplasm (Fig 3) The whole inclusion was always completely surrounded by a triple-layered membrane and did not seem to have any fixed position within the cytoplasm The inclusions contained *electron dense elements lipid bodies* and *fibrils* (Fig 4)

The electron dense elements appeared very different in size and shape Some of them were large (4 000–6 500 Å) mostly homogeneous round bodies with an even outline Apart from some small punched out light patches in the peripheral part in a few of them no details of the internal structure were revealed A bounding membrane was seldom observed around such elements that probably represented melanosomes The medium sized (about 1 000 Å diameter) electron dense elements were irregularly outlined ovoid bodies often with a granular appearance Finally numerous small jagged osmophilic granules (200–500 Å) were found lying mostly together in groups Some inclusions appeared studded with this material within limited regions

The lipid bodies were round elements that varied in diameter from 1 000 to 6 000 Å They showed no limiting membrane contained a substance which varied considerably in density from one body to the next and groups of the smaller ones were often filling up greater parts of the inclusions

The main part of the processes was made up by fibrils lying side by side in bundles that sometimes appeared twisted around the longitudinal axis (Fig 1) The fibrils showed an even thickness of about 90 Å they could be followed over large distances through the processes and they did not branch The single fibril was composed of two parallel filaments lying 30 Å apart (Fig 1)

The numerous inclusions lacking the characteristic ring shape contained the same material as described above but in some of them the fibrillar component appeared extremely scanty (Fig 5) Apart from the anterior border cells the ring shaped inclusions were observed in all parts of the iris stroma itself On the other hand they were found neither in the sphincter and the dilatator muscle cells nor in the pigment epithelial cells on the posterior iris surface Definite ring shaped inclusions were not observed in the iris from the 27 year old woman

Discussion

In the light microscope the inclusions of Biondi differ greatly in shape from one cell to another appearing like seal rings circles rackets rods and large granu

les Special staining procedures have revealed that they contain lipids and since they also show a positive Congo red reaction it has been suggested that they contain amyloid material as well (Biondi 1933 Bargmann 1955 Divry 1955 Dohrmann 1970 and Schwartz 1970) The complexity of these bodies have been confirmed by electron microscopic studies showing extremely electron dense granules of different sizes lipid droplets and fibers mixed together to a body which is separated towards the cell cytoplasm by a single limiting membrane. The fibrils form bundles which obviously run through large parts of the cell The positive Congo red reaction probably refers to the fact that the fibrils are doubled (Bargmann & Katritsis 1966 Oksche & Vaupel van Harnack 1969 and Oksche & Kirschstein 1972) The iris cell inclusions described in the present study showed the same variety in form as do the Biondi bodies and they also contained lipids electron dense granules including melanosomes as well as bundles of long doubled fibrils It should also be noted that they were observed in specimens from older individuals only although no definite conclusions can be drawn concerning this point since the present material includes only one specimen from a younger person Thus from a morphological point of view it seems reasonable to suggest that the Biondi bodies and the inclusions described in the present paper are identical

Several investigations have been undertaken in order to elucidate the structure and function of Biondi bodies of the choroid plexus and to the knowledge of the present author similar inclusions have not been described outside this region As previously reviewed by Davson (1956) the cerebrospinal fluid and the aqueous humour surrounding the choroid plexus and the iris respectively bear some obvious similarities to each other with regard to chemical composition On the other hand both of these fluids are produced by active secretion and accordingly they are different from plasma ultrafiltrate At present we can only draw attention to this fact without being able to state whether and to what extent development, distribution and function of the inclusions are influenced by environmental factors However the similarity in environment surrounding the tissues concerned as well as the aforementioned similarity of form between inclusions from the two regions makes it tempting to guess that the inclusions fulfil similar functions in the choroid plexus and in the iris

It has been pointed out (Fawcett 1966) that lysosomes are organelles that exhibit an almost unlimited diversity and there is considerable evidence that they are engaged in the removal of intracellular material (de Duve 1967) They are membrane bounded heterogeneous structures containing pigment masses dense bodies of different sizes lipid droplets and microbodies Furthermore they differ in appearance from one cell type or region to another *Apart from* the long fibrils the Biondi bodies as well as the inclusions demonstrated in the

present study correspond to the above summarized description of lysosomes If these organelles really are lysosomes it may be that the fibrils are derived from some degradation products being particularly abundant in some tissues only It should be added however, that cell organelles cannot be identified as lysosomes on the basis of their morphology alone

Acknowledgments

Sincere thanks are due to Professor Thore Lie Thomassen University Eye Department Rikshospitalet Oslo and to Professor Kristen Arnesen Department of Pathology Ullevål Hospital Oslo for placing specimens at my disposal Skilful technical assistance was provided by Mrs Ingerid Murer Knutzen

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ANTERIOR CHAMBER DEPTH IN GREENLAND ESKIMOS

I. A population study of variation with age and sex

BY

P. H. ALSBIRK

The remarkable prevalence of primary angle closure glaucoma (a.c.g.) in Greenland Eskimos called for a study of the anterior chamber depth (ACD) in this population. In the district of Umanaq the population above the age of seven was examined with a participation rate of 98%. Optical pachymetry was performed in 1 578 eyes of 931 Eskimos with Haag Streit 900 instruments.
As an introduction to further analyses the variability due to age and sex was studied. The results showed that the ACD increased through school age, achieved a maximum at puberty and decreased throughout adult life as in other populations. In contrast to previous large samples the variance of ACD increased significantly by age. No difference was found between boys and girls. In adults the ACD level was 0.16 mm shallower in females, i.e. a highly significant sex difference.

Key words: anterior chamber depth – population study – variation with age and sex – Greenland Eskimos

The clinical observation of frequent cases of acute glaucoma in Greenland Eskimos motivated the tonometric and gonioscopic screenings carried out in 1967–68. Prevalence studies showed that primary angle closure glaucoma (a.c.g.) was very common in this population and had contributed heavily to blindness (Clemmesen & Alsbirk 1969, 1971, Alsbirk 1970, 1973).

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Angle closure glaucoma is strongly associated with a shallow anterior chamber with narrow chamber angles In 1931 Rosengren showed convincingly that the axial anterior chamber depth in patients with earlier attacks of acute glaucoma deviated significantly from a sample of 810 Swedish controls As this shallowness was found even in the unaffected fellow eyes an anatomical predisposition was strongly suggested Through the last 40 years these findings have been confirmed in larger groups of a c g patients by means of optical and ultrasound methods (Törnquist 1956 Grieten & Weekers 1962 Lowe 1970 Storey & Phillips 1971 and others)

On account of this pronounced shallowness of the anterior chamber in Caucasians with a c g the distribution of this parameter in Eskimos with and without the disease seemed to be the first and most important quantitative parameter to study Therefore an extensive population survey was planned using the Haag Streit 900 instruments for depth measurements

In this introductory paper the distribution and variation of the anterior chamber depth (ACD) by age and sex will be documented

Material

The district of Umanaq in northern West Greenland (71°N) was chosen for this survey as useful information had already emerged from previous tonometric

Table 1
Population study of anterior chamber depth (ACD) in Umanaq Eskimos Sample size according to residence sex age and participation

Location	Total census population (all ages)		Selected for examination			Not examined		Examined	
	♂	♀	sex	age	no	diseased and objectors	absent	no	%
Umanaq town	442	439	♂ + ♀	7 +	672	8	3	661	98 4
8 villages	732	620	♂ + ♀	40 +	278	4	4	270	97 1
Umanaq district	1165	1059	♂ + ♀		950	12	7	931	98 0

and gonioscopic screenings in 1967 Biometric and genetic aspects of the survey called for an extension of the examinations to younger adults and children This was achieved in the town population of Umanaq where all inhabitants from the first school classes were examined in 1969 In the villages of the district all inhabitants above the age of 40 were examined

Table I shows the composition of the material under study Only 2 0 % (19/950) were not examined Five of the 19 persons refused to come

According to the census of December *31st 1968 just before the study the total population comprised 2 348 persons of which 5 3 %* (124) were ethnically Caucasians (Danes) registered as born outside Greenland Only the native population (2 224 persons) was used as basis for the present study This group is usually called Greenlanders while the term Eskimo is almost never used The appearance of the population however is predominantly Eskimoan with a certain Caucasian admixture clearly visible in all age groups *Historically* and genealogically this is well explained – chiefly *by the presence of* Danish administrators since 1763 when the colony in this area was founded However until recently relatively few Danes have lived in the area (e g in 1850 ?4/615 = 3 9 % 1915 16/1428 = 1 1 % 1930 18/1466 = 1 2 % 1945 37/1477 = ? 5 % 1960 50 ?013 = 2.5 % and 196? 85/??34 = 4 0 %)

In this study the designation Eskimo indicates a person with at least one parent belonging to the well defined socio cultural group of Greenlanders Thus even 50 % hybrids with a known *or unknown Danish father and a Greenland mother* were included but they were probably less than ten No person with a Greenland father and a Danish mother appeared

Table II

ACD measured persons and eyes without impeding changes with deficit specified

ACD measurable in		No of persons	No of eyes	
right eye	left eye		measured	*not measured*
	+	900	1800	–
	–	8	8	8
–	+	?	?0	20
–	–	3	–	6
Total		931	*18 S*	34*

An phthalmia ? aphakia 5 aquired deformity 22 congenital *malformation* 5 acute changes 2 total 34 eyes.

Angle closure glaucoma is strongly associated with a shallow anterior chamber with narrow chamber angles In 1931 Rosengren showed convincingly that the axial anterior chamber depth in patients with earlier attacks of acute glaucoma deviated significantly from a sample of 810 Swedish controls As this shallowness was found even in the unaffected fellow eyes an anatomical predisposition was strongly suggested Through the last 40 years these findings have been confirmed in larger groups of a c g patients by means of optical and ultrasound methods (Törnquist 1956 Grieten & Weekers 1962 Lowe 1970 Storey & Phillips 1971 and others)

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Umanaq district	1165	1059	♂ + ♀		950	12		931	98 0

♀ age groups									♂ all ages	♀ all ages
7–9	10–14	15–19	20–29	30–39	40–49	50–59	60–69	70 +	7 +	7 +
										1
	1								2	1
	1								4	4
	1	2	1						5	3
1	1	1							14	6
1		4		1					29	20
2	3	5	7		1				41	26
4	6	8	5	2		1				
9	11	7	10	2		2			53	41
7	5	8	10	7	3	2	1	2	45	45
8	9	6	6	2	6	2	2		57	41
5	5	2	6	4	11	4	1		40	38
1	4	1	3	6	8	2	7	1	34	33
2		3	3	11	10	5	6	2	29	42
	1		2	9	12	7	7		32	38
		1		6	9	4	3		24	23
			6	2	7	4	[illegible]	2	18	28
				2		7	2	2	11	20
				1	3	7	3	5	11	19
				1		4	4	2	5	11
					1	2	6	1	4	10
						3	4	2	4	9
					1		3		1	4
							1			1
							1			1
40	50	43	59	[illegible]	[illegible]	56	58	19	463	465
8.0	11.7	17.1	24.8	35.1	43.5	54.2	63.5	74.2	35.3	36.0
2.85	2.90	2.94	2.[illegible]7	2.51	2.39	2.[illegible]4	2.13	2.11	2.68	2.54
0.[illegible]0	0.27	0.[illegible]6	0.31	0.[illegible]9	0.2	0.36	0.38	0.37	0.38	0.42
0.011					-0.016					
0.014					0.001					

Table III
Distribution of ACD in 928 Umanaq Eskimos by sex and age

ACD mm		♂ age groups 7–9	10–14	15–19	20–29	30–39	40–49	50–59	60–69	70+
3 60–3 69										
3 50–3 59			1	1						
3 40–3 49		1	1	1	1					
3 30–3 39				4	1					
3 20–3 29		1	4		8	1				
3 10–3 19		6	11	4	6	1		1		
3 00–3 09		5	16	4	4	4	4	3	1	
2 90–2 99		9	14	4	13	7	4		2	
2 80–2 89		8	9	1	9	6	4	7		1
2 70–2 79		11	7		8	7	13	7	4	
2 60–2 69		1	5	5	5	6	8	5	3	2
2 50–2 59		2	2	1	5	4	8	9	3	
2 40–2 49		1	1			3	9	9	5	1
2 30–2 39		1		1	1	2	12	6	7	2
2 20–2 29			1			1	8	3	7	4
2 10–2 19					1	1	3	5	8	
2 00–2 09							1	5	3	2
1 90–1 99							2	4	3	[illegible]
1 80–1 89						1		3		1
1 70–1 79								1		3
1 60–1 69									3	1
1 50–1 59									1	
1 40–1 49										
1 30–1 39										
1 20–1 29										
1 10–1 19										
No of persons		46	72	26	62	44	76	68	50	19
Age mean	$\bar{x}$	8 1	11 7	17 0	24 8	34 1	43 9	54 2	63 9	75 2
ACD mean	y	2 89	2 95	3 00	2 91	2 72	2 54	2 44	2 31	2 15
ACD stand dev	s_y	0 21	0 21	0 30	0 25	0 28	0 26	0 33	0 33	0 34
regr coeff	b_{yx}	0 022				−0 015				
stand dev of b_{yx}	s_b	0 009				0 001				

sexes (skewness ± standard error for 463 ♂ −0.48 ± 0.11, 465 ♀ −0.32 ± 0.11). However when children and adults were treated separately this skewness disappeared. No significant deviation from normality was found in any single or combined age subgroup of Table III, the probability levels ranging for $P < 0.95$ to $P < 0.2$. On this background the ordinary statistical parameters shown in Table III were the tools of choice.

Age variation of the ACD distribution

The influence of age on ACD appears from the distributions and the age group means in Table III. In the Figure these mean values are shown with double arrows indicating 95 % confidence limits. The fluctuation of mean values with a maximum near puberty and declining throughout adult life is clearly demonstrated.

Evidently the material was so large that lines traced through the mean values of the single age groups were fairly smooth as the preliminary reports have shown (Alsbirk 1973, Alsbirk & Forsius 1973). The maximum around puberty appeared in both sexes. Linear regression coefficients of ACD (y) on age (x) were calculated below and above age 15 (Table III) as the simplest estimators of this age dependency.

In the 208 *children* the pooled data of both sexes displayed a positive regression coefficient on age ($b_{yx} \pm s_b = 0.019 \pm 0.007$) achieving a significance level of $P < 0.01$. As no significant difference was found betwen boys and girls in variances, means and slopes, this combination seemed justified. In the separate sex groups only the boys' slope differed significantly from zero ($P < 0.05$).

In *adults* the reduction of ACD with increasing age was very pronounced already in the twenties and continued through the age groups with an overall rate of about 0.15 mm per decennium.

As the age group means of the Figure show, this reduction seemed to be greater in younger than in older adults. In order to allow a later comparison with elderly Eskimos from other districts a subdivision at the age of 40 was carried out. Using this arbitrary separation the regression coefficients in female adults below and above 40 were found to be −0.023 ± 0.003 and −0.011 ± 0.002, i.e. a highly significant difference ($t = 3.3$, $P < 0.001$). For males the corresponding slopes were − 0.017 ± 0.004 and − 0.011 ± 0.002, this difference being insignificant.

The variance of ACD – indicated in Table III by its square root, the standard deviation (s_y) – seems also to be influenced by age. As an increase in the variance of ACD with age might have important causative aspects in relation to a.c.g., a statistical evaluation was carried out.

The coefficient of variation was found to double in 50 years (age 15 to age 65), in females from 8.4 to 17.3 % and in males from 7.7 to 14.5 %.

A specification of the number of ACD measured eyes in the 931 persons is shown in Table II In the great majority 96 7 % both eyes were measured From 28 persons (3 0 %) only one eye was included in the sample and three persons could not be measured in any eye The reasons why 34 eyes were excluded are given below the Table

Method

Haag Streit slit lamp 900 with two attachable pachymeters for depth measurement no I and no II was used throughout the procedure recommended by the manufacturers being strictly observed Every eye was measured by three readings with both instruments No I for corneal thickness (read to nearest 1/100 mm unit) and no II for anterior chamber with cornea included (read to nearest 1/20 mm unit) The ACD value of the eye appeared as the difference between mean II and mean I

Details of the method including error of measurement correction values due to radius of corneal curvature and side difference with left eye preponderance will be given elsewhere The variability due to these sources was found to be very small compared with the influence of age and sex described in this paper

Statistical analyses were mainly carried out by computer programs working on individual not on grouped data where nothing else is stated

Results

The ACD results from the 928 Umanaq Eskimos in this population study are presented in Table III

The right and left ACD values were found to be highly correlated (r = 0 98 for 900 pairs of eyes) As this source of variation was found to be so small the interpersonal variation became the main subject to be studied It was analysed by use of either the average of both eyes or the single eye value when only one eye was measured Therefore all numbers in Tables and Figure refer to persons not to eyes

Chi square goodness of fit tests for normality showed that only the total distributions differed significantly from a Gaussian curve (♂ $P < 0 01$ ♀ $P < 0 05$) In these all age groups a skewness towards the smaller ACD values was found to be significant in both

sexes (skewness ± standard error for 463 ♂ −0.48 ± 0.11, 465 ♀ −0.32 ± 0.11). However, when children and adults were treated separately this skewness disappeared. No significant deviation from normality was found in any single or combined age subgroup of Table III, the probability levels ranging for $P < 0.95$ to $P < 0.2$. On this background the ordinary statistical parameters shown in Table III were the tools of choice.

Age variation of the ACD distribution

The influence of age on ACD appears from the distributions and the age group means in Table III. In the Figure these mean values are shown with double arrows indicating 95% confidence limits. The fluctuation of mean values with a maximum near puberty and declining throughout adult life is clearly demonstrated.

Evidently the material was so large that lines traced through the mean values of the single age groups were fairly smooth as the preliminary reports have shown (Alsbirk 1973, Alsbirk & Forsius 1973). The maximum around puberty appeared in both sexes. Linear regression coefficients of ACD *(y) on age (x)* were calculated below and above age 15 (Table III) as the simplest estimators of this age dependency.

In the 208 *children* the pooled data of both sexes displayed a positive regression coefficient on age ($b_{yx} \pm s_b = 0.019 \pm 0.007$) achieving a significance level of $P < 0.01$. As no significant difference was found betwen boys and girls in variances, means and slopes this combination seemed justified. In the separate sex groups only the boys' slope differed significantly from zero ($P < 0.05$).

In *adults* the reduction of ACD with increasing age was very pronounced already in the twenties and continued through the age groups with an overall rate of about 0.15 mm per decennium.

As the age group means of the Figure show, this reduction seemed to be greater in younger than in older adults. In order to allow a later comparison with elderly Eskimos from other districts a subdivision at the age of 40 was carried out. Using this arbitrary separation the regression coefficients in female adults below and above 40 were found to be −0.023 ± 0.003 and −0.011 ± 0.002, i.e. a highly significant difference ($t = 3.3$, $P < 0.001$). For males the corresponding slopes were −0.017 ± 0.004 and −0.011 ± 0.002, this difference being insignificant.

The variance of ACD – indicated in Table III by its square root, the standard deviation (s_y) – seems also to be influenced by age. As an increase in the variance of ACD with age might have important causative aspects in relation to a.c.g., a statistical evaluation was carried out.

The coefficient of variation was found to double in 50 years (age 15 to age 65) in females from 8.7 to 17.3% and in males from 7.7 to 14.5%.

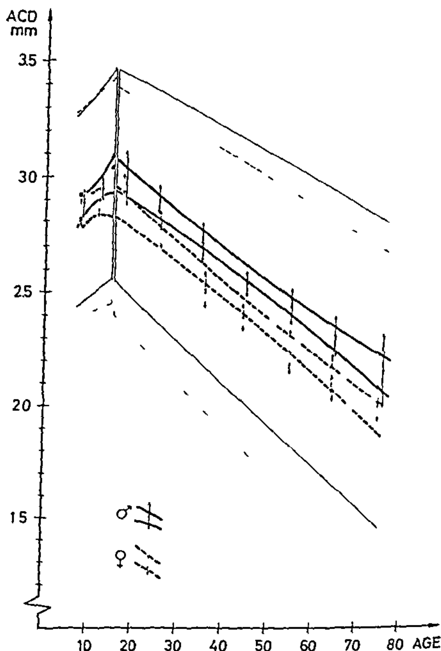


Fig 1

Variation of ACD with age and sex in an Eskimo population (208 children and [illegible]0 adults) The *mean values* with 95 % confidence limits are indicated by *a)* double arrows in nine groups and *b)* by linear regression intervals in children and adults (heavy hyperbolic curves) The increasing *variance of ACD* with age is outlined by the 95 % confidence limits of the individual ACD values (thin lines)

A Spearman rank correlation test of s_y^2 and age based on the 9 age groups was significant with respect to the 0 01 level in both sexes

Table IV summarizes the special type of analysis of variance of this ACD variance (s_y) carried out according to a method proposed by R. Fischer as used by Fraser Roberts and Mellone (195) on distributions of intelligence and by Hamilton et al (1954) on blood pressure distributions

The mean squares due to linear regression and due to remainder i e variation about regression and between age groups were tested by F tests using a theoretical variance of variance measure calculated according to the first paper cited For both sexes the linear regressions had a positive slope and seemed to fit the data sufficiently well as the remainders turned out to be far from significant The estimating equations are shown below Table IV

Table IV

Analysis of variance of ACD variance (s_y^2) in 9 age groups of the Umanaq sample subdivided according to sex

Source	Degrees of freedom	Weighted sum of squares	Mean square (1)	Theoretical variance (Df = ∞) (2)	F ratio between (1) & (2)
463 ♂ 9 age groups					
Due to linear regression	1	0.231	0 231	0 012	19 7 $P < 0 01$
Remainder	7	0 067	0 010	0 014	0 7 n.s
Total	8	0 298			
46 ♀ 9 age groups					
Due to linear regression	1	0.340	0 340	0 017	20 1 $P < 0 01$
Remainder	7	0 126	0 018	0 019	0 9 n.s
Total	8	0 466			

Linear regression of ACD variance on age (x)

♂ $s_y^2 = 0\ 0379 + 0\ 0011\ x\ (s_b = 0\ 0002)$

♀ $s_y^2 = 0\ 0427\quad 0\ 0014\ x\ (s_b = 0\ 0003)$

As a consequence of this significant age association the smoothed increasing variance estimates were included in the Figure represented by the 95% confidence interval of the distribution of individual ACD values (mean ± 1 96s_r)

Thus the relationship between age and ACD in adults involved two combined changes of the ACD parameters decreasing mean and increasing variance

The relationship between sex and ACD

When the variation due to age was accounted for by appropriate linear re gressions it became possible to compare the main effect of sex on ACD in the sample

In children the sex difference was far from significant (0 04 ± 0 03 mm)

In the 720 adults on the contrary the sex difference was found to be extremely significant when the linear regression intervals were compared (0 16 ± 0 02 mm t = 6 7)

The relative shallowness in females apparently became increasingly pronounced with age but the slopes did not differ significantly between the sexes either in the total adult group or within the two age groups below and above 40

The *variance of ACD* increased with age with the greater slope in females (Table IV) but this sex difference was non significant At the general mean age 35 6 years the males had a variance of 0 0770 mm the female value being 0 0916 mm corresponding to the standard deviations 0 28 and 0 30 mm respectively The ratio between the adjusted mean variances did not achieve significance at the 0 05 level by a two tailed test (F = 1 19) Correspondingly no significant sex difference was found in any of the three greater age groups representing children younger and older adults

Thus it might be concluded that the shallowness of the anterior chambers was definitely more pronounced in female adults while the sex difference was insignificant in children In the variances no difference was demonstrated

Discussion

An extensive population study of the ACD distribution in Eskimos has not been accomplished before the present survey With a 98% participation rate the population study of about 900 persons should be able to delineate the age and sex pattern of this parameter thus contributing particularly to the Eskimoan and also to the more general field of ophthalmo anthropology

The influence of *age* on the ACD distribution did not seem to differ funda

mentally from the growth patterns of the anterior segment mainly known from Caucasians and Japanese Most studies however have only comprised either children or adults

The increase of ACD in *children* was one of the characteristics of the large refraction component study carried out by Sorsby et al (1961) By ophthalmo phakometry of 1 345 children *3–13 years old they found positive slopes* (0 012 in boys and 0 019 in girls) in good agreement with the findings in Eskimo children

Delmarcelle & Luyckx Bacus (1971) published the first large pachymetry study of ACD in Belgian children measuring about 1 600 non cycloplegic eyes *with the same technique* as I used Their results however were not derived from a proper population study as the material was selected according to the refraction of the single eyes Their data confirmed the increase of ACD in the children examined above the age of two Hypermetropics had a maximum at about 10 years while the ACD of emmetropics and myopics seemed to increase for another 5 years in agreement with the present population study

Ultrasound oculometry studies of the sagittal growth of the eye in children *confirm these findings After a review of the literature concerned* Larsen (1971a) showed that the ACD increased by 1mm through the first year and a half by 1/3 mm through the next 6 years and finally by 1/10 mm until the age of puberty He examined 931 Norwegian children from newborn to 14 years old and showed that this increase reflected the flattening of the lens and growth of the axial length of the bulb until puberty (Larsen 1971b c d)

Young & Leary (1973) studied the biometric pattern of Alaskan Eskimos by *ultrasound In a population study of 344* persons they found that ACD and lens thickness showed little or no change between the ages 2 and 25 years while the oldest age group (mean age 58) had ACD values about 0 9 mm below the adolescents

Perez Llorca Rodrigo (1971) examined 1 483 persons (Spaniards) from pre mature newborn children to 80 year old people *Even if his ultrasound data* were very briefly presented the results gave evidence of an ACD maximum just after puberty followed by a decrease of about 0 9 mm through the next 60 years

The decreasing ACD in *adults* has been a well known fact for more than 50 years Raeder (1922) found a nearly linear reduction of ACD *with age amount* ing to about 0 65 mm in 50 years Therefore he stated that it would not be justifiable to specify *any single valuable mean of ACD* Rosengren (1931) and Tornquist (1953) demonstrated the decrease with age of ACD measured optic ally *in* large control samples collected for studying ACD in patients with previous acute glaucoma Their control curves showed a faster decrease with

age in younger adults than later in life Törnquist stated that a quadratic parabola most closely fitted his material of 398 Swedes ACD (mm) = 3 55–0 0295x + 0 000133x^2 (x = age) the second degree term accounting for the deceleration of the ACD reduction with age in the older groups

The use of curvilinear regression statistics was not attempted in the present study, but the mean values of the adult age groups with the more horizontal slope in older Eskimos corresponded to these findings

The *ACD variance* was shown to increase with age in the material presented This finding seems to be new When calculating the variance in the 11 five-year groups of Rosengren s 810 controls no evidence of an increase was found as the result of a Spearman rank correlation test was far from significant but no children or adolescents were represented The same applies to Tornquist s sample (1953) and to the all-age Japanese sample (n = 435) examined by Nakajima et al (1968) The increasing variance demonstrated in the Umanaq sample implies a growing interindividual heterogeneity with increasing age Whether this finding applies to Eskimos only is still an unsolved question

The *sex difference* of ACD was found to be significant in *children* in the above mentioned large studies of Sorsby et al (1961) Delmarcelle & Luyckx Bacus (1971) and Larsen (1971a) with the anterior chambers nearly 0 1 mm shallower in girls The 208 school children from Umanaq showed a corresponding but not significant difference (0 04 mm)

The relative shallowness of ACD in *adult* females was evident and proved to be significant in this sample Tornquist (1953) found a corresponding difference which however did not achieve statistical significance at the adopted rather extreme level ($t \geq 3\ 0$ $P \leq 0\ 0027$) The sex difference found by Tornquist was 0 09 ± 0 035 mm and the difference in Rosengren s material was 0 08 ± 0 023 mm calculated by Tornquist (1953) A similar difference appeared in the study by Calmettes et al (1958) where however no statistical evaluation was attempted

The sex difference in Eskimo adults was highly significant larger than the above Caucasian values However the samples were collected in different ways The Swedish results were based on combinations of various non ophthalmic hospital patients ophthalmic outpatients medical personnel and others no patients with diseases which might influence the anterior chamber being included

The Eskimo results were collected in total population groups as described in order to cover the total variability in this population Thus the sample from Umanaq included 22 a c g patients (20 ♀ + 2 ♂) with previously known a c g But even in a special subsample of 843 Umanaq Eskimos from which the a c g patients their sibs and children and every person with impeding changes even

in one eye were rejected the sex difference was highly significant 0 12 ± 0 026 mm i e only a little less than the overall 0 14 mm sex difference stated in Table III In a following paper the probable association between this sex difference and the female preponderance in all samples of a.c.g patients will be further elucidated

In the present paper no attempt was made to compare the *level of ACD* in Umanaq Eskimos with that of other populations the age and sex variability being the main topic However as preliminary reports have shown (Clemmesen & Alsbirk 1971 Alsbirk & Forsius 1973) the very low level of the ACD distribution *in Umanaq Eskimos immediately attracted considerable attention in relation* to the highly prevalent a c.g in this population

On this background the variation of ACD in older Eskimos of other districts became a most important subject to study A subsequent paper presents an analysis of the geographical variation of ACD with comparisons between Eskimos *and other ethnic groups*

Acknowledgements

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ANTERIOR CHAMBER DEPTH IN GREENLAND ESKIMOS

II Geographical and ethnic variation

BY

P H ALSBIRK

A population study of the anterior chamber depth (ACD) was performed in 1,5‹5 Greenland Eskimos using optical pachymetry (Haag Streit 900) From six separate districts 1 0 ? Eskimos over the age of forty were examined The participation rate ranged *from 82–98 %* *The geographical* variation was found to be relatively small No difference between the unmixed east coast Eskimos and the mixed west coast Eskimos could be demonstrated

From one of the towns 503 Eskimos aged 7–39 years were examined For ethnic comparison 87 adult Danes living in Greenland were measured as controls Their ACD level was found to be much higher than in Eski*mos clo ely agreeing with other Caucasian samples*

The pronounced shallowness thus observed in Eskimos from all parts of Greenland has also been found recently in Canadian and Alaskan Eskimos A shallow anterior chamber seems to be an important characteristic f Eskimos

Key words anterior chamber depth – East/West Greenland Eskimos – shall w anterior chambers – Danish controls – pachymetry

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The background of the present survey was the high prevalence of angle closure glaucoma (a c g) in Greenland Eskimos This challenge to the health service called for a study of the most relevant oculometric parameter the anterior chamber depth (ACD) In a previous paper the results of a population study in Umanaq was presented (Alsbirk 1974) A total of 931 Eskimos 95 % of the age groups over 7 years were examined by optical pachymetry (Haag Streit 900) The variation of ACD with age and sex was found to be highly significant. After a maximum at puberty the mean of ACD decreased while the variance of ACD increased in both sexes Adult females had shallower chambers than males.

When compared with earlier Caucasian materials (e g Tornquist 1955) a very low level of ACD in Umanaq Eskimos was immediately suspected Therefore some very important questions arose *a)* Are the shallow anterior chambers a characteristic of all Eskimos in Greenland? *b)* Does the level of ACD in Eskimos differ from a Caucasian control sample living in Greenland and examined by the same method? *c)* Do these controls agree with other Caucasians? – This paper tries to answer these questions

Material

The geographical variation of ACD was studied in the population groups listed in Table I the order being from East Greenland through southern to North West Greenland (Fig 1) The Greenland Eskimos total 39 000 and are located in 17 towns and about a 100 villages widely scattered along an arctic coast of 5 000 km Therefore certain *locations* had to be selected for the study

The East Greenlanders form the purest Eskimo group (Skeller 1954) A sample was collected in 1970 in Angmagssalik which is the most accessible of the two districts The mixed population of West Greenland was examined in five separate locations typical of the different regions The population in the district of Umanaq had been examined in 1969 (Alsbirk 1974) The three town samples to the south of Umanaq were examined in 1970

In Upernavik which is adjacent to Umanaq but very isolated an extremely shallow ACD level had previously been found by Forsius et al (1970) This prompted a reexamination of the population which I performed in 1972 As it was found that the *extraordinarily* low level was solely due to a methodological problem (Alsbirk & Forsius 1973) the results were included in the present analyses

Table I
Population study of anterior chamber depth (ACD) in Greenland Eskimos. Sample size according to residence, sex, age and participation

Location		Total census population (all ages)		Selected for examination			Not examined		Examined	
		♂	♀	sex	age	no	diseased and objectors	absent	no	%
Angmagssalik (Kap Dan & Kungmiut incl.)	(L)	86	876	♂ + ♀	40+	219	7	29	183	84
Julianehåb	(W)	1043	1128	♀	40+	211	13	6	192	91
Sukkertoppen	(W)	1030	1034	♀	50+	113	9	11	93	82
Egedesminde	(W)	1370	1442	♀	50+	126	6	9	111	88
Umanaq district	(W)	1165	10 9	♂ + ♀	7+	950	12	7	931	98 0
Upernavik (Augpilagtoq incl.)	(W)	447	408	♀	40+	68	–	3	63	96
Total		5901	6007			1687	47	65	1575	93 4

L East Greenland W West Greenland

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Angmagssalik (Kap Dan & Kungmiut incl.)	(E)	786	876	♂ + ♀	40+	219	7	29	183	84
Julianehåb	(W)	1043	1198	♀	40+	211	13	6	192	91
Sukkertoppen	(W)	1090	1034	♀	50+	113	9	11	93	82
Egedesminde	(W)	1370	1442	♀	50+	196	6	9	111	88
Umanaq district	(W)	1165	1059	♂ + ♀	7+	950	12	7	931	98.0
Upernavik (Augpilagtoq incl.)	(W)	447	408	♀	40+	68	–	3	65	96
Total		5901	6007			1687	47	65	1575	93.4

E East Greenland W West Greenland



Fig 1
Map of Greenland with the locations of the ACD survey indicated

The clinical aspect will be disregarded in this paper However within the locations the selection according to sex and *age* was made with a view to finding the maximum number of persons with a risk of a c g Therefore in the districts outside Angmagssalik and Umanaq only the *females* were examined because the usual female preponderance in the a c g sex ratio had been recognized in Greenland as elsewhere (♀/♂ = 68/26 Clemmesen & Alsbirk 1969) Correspondingly the age pattern of known a c g patients at the time of diagnosis had suggested that *40 years* would be a reasonable lower age limit The short time available for the study in two districts necessitated restriction to the 50+ groups

Every Eskimo registered in these *locations* belonging to the selected sex and age *groups* was *invited to attend* the examination by an explanatory letter The term "Eskimo" is used in the wide sense earlier described (Alsbirk 1974) in West Greenland including native Greenlanders with discernable Caucasian admixture However in East Greenland 10 immigrants from the west coast were excluded in an attempt to preserve the group as a pure East Greenland Eskimo sample.

The percentage of persons not examined was only 2 0% in Umanaq and averaged 1?6 / in the five other districts where the short time available prevented the survey from catching the last few Temporary absence due to hunting fishing or visits to other districts accounted for more than half of the deficit while only six persons directly refused to participate

Thus the total number of Eskimos examined amounted to 1,575 The *great majority* 96 1% had both eyes *measured* the average value of the two eyes being used in further calculations Fifty six persons (3 6%) had only one eye included in the sample and five persons could not be measured in any of their eyes In this way 1 5/0 persons remained for the study of the *interpersonal variation* Anophthalmia (12 eyes) aphakia (6) aquired deformity (37) congenital malformation (?) acute changes (?) and insufficient fixation (?) were the reasons for exclusion of the 66 eyes

Eighty seven adult *Danes* living in Umanaq constituting about 90% of the non Eskimo population present in 1969–70 were examined as a Caucasian control sample They were mainly workmen clerks teachers and housewives who had been in Greenland for a few months or years

Methods

Haag Streit 900 pachymeters no I (*for corneal thickness*) and no II (for ACD with cornea included) were used for Umanaq Eskimos and Danes according to the manufacturer's *instructions In the five other districts* a time saving modification was used Both distances were measured with pachymeter II with three readings as before A slight reduction in the precision of corneal thickness measurements was *considered unimportant* in relation to the main purpose ACD measurements Details concerning the method will be presented later All measurements were made by the author

The statistical analyses were carried out by computer programs in the following steps

a) Analyses of the ACD distributions by chi square goodness *of fit* tests as previously done in the Umanaq material (Alsbirk 1974)

b) When no significant deviation from normality was found parametric methods were preferred The age composition of the samples being slightly different adjustment by age had to be used in all comparisons Linear regression parameters of ACD (y) on age (x) were estimated within each location and sex group (e g Table II) By these estimating equations the ACD mean values (ŷ) with their standard errors or confidence limits could be given at any age (e g Table IV Figs 2 and 3)

c) A final comparison between several location groups necessitated the use of an analysis of covariance on age As prerequisites the homogeneity of residual variances ($s_{y.x}$) was tested by an F test (2 ♂ samples) or a Bartlett s homogeneity of variance test (6 ♀ samples) Regression coefficients were compared by F tests (e g Table III) or t tests

d) Comparison with other samples from the literature was rather complicated as the regression parameters of ACD on age were mostly lacking in the presentations The estimating equations had to be calculated based on the relevant age group means and standard deviations of ACD Certain Swedish Belgian and Japanese samples were made – roughly – comparable with my data in this way (e g Table IV Fig 3)

Results

Geographical variation of ACD in Greenland Eskimos

In Table II the ACD parameters of two male and six female groups are given Gaussian distributions were accepted as no deviation from normality was found in any of the single or combined groups (the probability levels ranged from 0 95–0 1)

The two *male* samples representing East and West Greenland Eskimos were very similar also when adjusted by age but the variances ($s_{y.x}$) differed significantly ($P < 0\ 01$)

In Fig 2 a comparison is given for the *female* samples adjusted to the age of 55 near the general mean A considerable overlap even between the geographically most separated groups was evident (e g Upernavik and Angmagssalik) The variances in these elderly female groups were found to be homogeneous and the increase with age was not significant although this trend had previously been found in Umanaq when children and younger adults were included (Alsbirk 1974) Under these conditions the various ACD mean levels (Table II) were compared as shown in Table III

The six regression coefficients did not differ from each other as demonstrated in lines no (1) to (3) of Table III thereby justifying the pooling of all groups as in line no (1)

Table II

ACD ampl par meters from 1 0(7 elderly Greenland Eskim(s according to sex and location

Sex	Di trict		Age	No examined n	Mean age x	Mean ACD y (mm)	Stand dev s_y (mm)	Kegr coeff b_r (mm)	Stand dev about linear regr $s_{y x}$ (mm)
♂									
	Ai gmags lik	(E)	40	8J	50 6	2 44	0 .6	-0 01?	0 ?4
	Umanaq	(W)	40	213	54 7	2 4?	0 33	-0 011	0.31
♂	Total			30?	53 5	2 43	0 31	-0 011	0 2J
♀									
	Angmagssalik	(E)	40+	94	51 8	2 34	0 31	-0 007	0 30
	Julianehåb	(W)	40+	192	53 5	? 30	0 31	-0 015	0 ?8
	Sukkertoj pen	(W)	50+	93	62 4	2 34	0 31	-0 007	0 30
	Egedesminde	(W)	50+	111	61 3	2 2	0 30	-0 010	0 ?J
	Umana j	(W)	40+	21?	54 6	2 25	0 35	-0 011	0 34
	Upernavik	(W)	40+	63	55 1	2 33	0 35	-0 011	0 33
♀	Total			765	55 9	2 29	0 33	-0 010	0 31

L Fast Greenland W West Greenland

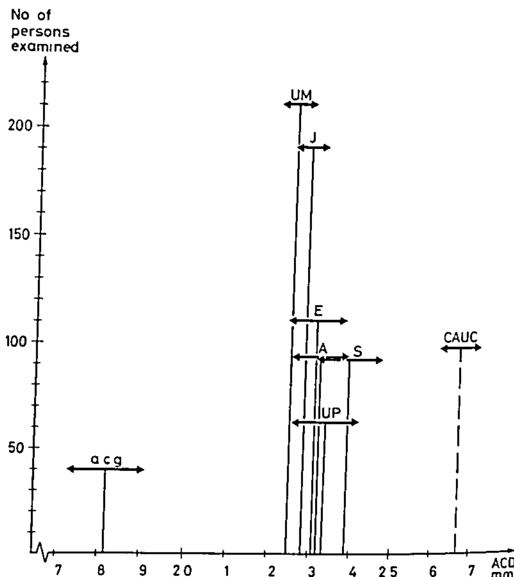


Fig 2

Variation of ACD with location in 765 elderly female Eskimos from 6 separate districts The horizontal double arrows show ACD means with 95% confidence limits adjusted to age 55 separated vertically according to sample sizes UM Umanaq J Julianehåb E Egedesminde A Angmagssalik UP Upernavik and S Sukkertoppen CAUC adjusted mean of 98 Caucasian women of ages 50 & 65 (Tornquist 1953) a c g 40 female Eskimo a c g patients as at age 55

On the contrary the differences between the level of the six paralleled regression lines accounted for a large value of deviation sum of squares (line no (6) as (5) minus (1)) As the F ratio shows the 6 ACD levels do differ The comparison which was considered important *a priori* – East versus West Greenland Eskimos – turned out to be far from significant (7) The rank of mean values in Fig 2 called for an *a posteriori* test of the

Table III

Analysis of covariance of ACD on age in female samples from six districts with analys s of variance between slopes and adjusted means

Source of variat on	Degrees of freedom d f	Age (x) sum of squares xx	Sum of products xy	ACD (y) sum of squares yy	Deviation about regression				
					d f	sum of squares y y*	mean square	F ratio	proba bility
(1) With n 6 samples pooled	759	73825	– 68 7	79 34	758	71 34	0 0941		
(2) Within 6 y y summed					753	71 03			
(3) Within, due to difference between 6 slopes					5	0 31	0 06 0	0 7	n s
(4) Between 6 samples	5	10296	– 16 5	1 11					
(5) Total	764	84121	–785 2	80 45	763	73 12			
(6) Difference between 6 adjusted means					5	1 78	0 36	3 79	$P < 0.01$
(7) East versus West Greenlanders					1	0 014	0 014	0 14	n s
(8) Difference between 5 adjusted means (Sukkertoppen excluded)					4	0 59	0 15	1 55	n s

( ) indicates line no

differences between the groups By a Student Newman Keuls procedure only the differences between Sukkertoppen versus Umanaq ($P < 0.01$) and between Sukkertoppen versus Julianehåb ($P < 0.05$) were found to be significant while the five groups outside Sukkertoppen could not be shown to differ cf the F test in line (8)

In conclusion the study of geographical variation showed that East Greenland Eskimos had an ACD level in agreement with the mixed Eskimos of the west coast samples One of these however deviated significantly towards a higher level

The ACD level of Danes in Greenland compared with Eskimos

The Danes in Umanaq made up a suitable control sample for methodological and ethnic comparisons Table IV gives the results for 87 adult Danes (48 ♂ 39 ♀) No significant sex difference was found The relevant Eskimo sample for comparison was the 720 Umanaq Eskimos over 15 years previously described (Alsbirk 1974) When adjusted to the mean age of the Danes 31 5 years the Eskimo mean ACD was 0 24 mm *below* the mean of the Danes i e a very pronounced ethnic difference ($t = 6.4$ $P \leqslant 0.001$) The other samples of Table IV will be compared and discussed below

Discussion

In answer to the questions in the introduction two results have turned up so far The ACD distributions were found to be fairly uniform in various Eskimo populations from all parts of Greenland Furthermore the Danish control sample had significantly deeper chambers than the Eskimos Thus a relative shallowness of the anterior chambers in *Greenland Eskimos* seemed to exist.

The Greenlanders constitute about half the total of all Eskimos In *Canada* and *Alaska* the Eskimos total about 17 000 and 28 000 respectively Recently convincing information about their ACD levels has come to light from two sources

Forsius examined the populations in two small communities Igloolik in Canada and Wainright Alaska The preliminary findings together with some of the results from Greenland all obtained by the same method were published by Alsbirk & Forsius (1973) A striking similarity was found In Table IV and

Table II

ACD linear regression parameters of adult Danes and Greenland Eskimos examined in the same way compared with Swedes (Tornquist (1953)) Two Eskimo samples from Canada and Alaska and a Mongoloid sample are included

Ethnic group	age	No of examined n	Mean age x	Mean ACD with standard error $y \pm s_y$ (mm)	Regr coeff b_{yx} (mm)	Stand error of b_{yx} s_b	Stand dev about regr $s_{y.x}$
Danes	15	87	31.5	2.94 ± 0.035	−0.016	0.004	0.32
Swedes (Tornquist 1953)	20+	398	47.4	2.86 ± 0.015	−0.011	0.0009	0.30
at age 31.5				*2.98 ± 0.018*			
Umanaq Eskimos	15+	790	43.0	2.53 ± 0.012	−0.015	0.0007	0.3
at age 31.5				*2.70 ± 0.014*			
Canadian Eskimos (Forsius 1971)	1[illegible]+	181	32.4	2.61 ± 0.0[illegible]2	−0.0[illegible]3	0.002	0.30
Alaskan Eskimos (Forsius 1971)	15+	55	37.5	2.69 ± 0.050	−0.016	0.003	0.37
Japanese (Nakajima et al 1968)	15+	337	43.7	2.42 ± 0.017	−0.016	0.001	0.32

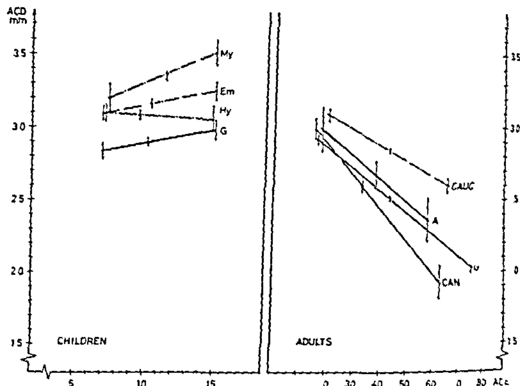


Fig 3

ACD and age in Eskimos and Caucasians Linear regressions are shown with 95% confidence limits at mean ages (↕) and at arbitrary extreme ages (↕)

– – – CAUC (Caucasians) *Children* selected by refraction as My 245 myopic eyes (mean refraction – 3.9 D) Em 457 emmetropic eyes (± 0 25 D incl) and Hy 562 hypermetropic eyes (+ 3 0 D) (Delmarcelle & Luyckx Bacus 1971) *Adults* from Törnquist (1953)

——— *Eskimos* from G Umanaq *Greenland* A Wainwright *Alaska* and CAN Igloolik *Canada* (Sample sizes are given in Table II)

Fig 3 the results from Umanaq Igloolik and Wainwright adults are shown (the sexes combined) The regression of the Alaskan group closely parallels the Umanaq level while the Canadian sample is different, the slope being significantly steeper Thus the older Igloolik Eskimos had much shallower chambers than Greenlanders while the youngest adults (and children) tended to have deeper chambers

A similar finding was made by Drance et al (1973) in a population study at Eskimo Point and Coral Harbour in Canada with the same method In 57 Eskimos they obtained the regression ACD (mm) = 3 634–0 0235 x (x = age) Thus the slope agreed with the Igloolik sample but the ACD level was as much as 0 26

mm higher This difference seems to be highly significant even if only a rough statistical estimate was possible with the data given.

Thus in spite of certain discrepancies and small samples a rather uniform low level of ACD seems to be a characteristic of the Eskimos all over their vast arctic territory

Other ethnic groups in the arctic have been examined with the same technique. Forty four *Indians from Youkon living in the same* northern latitude as the Eskimo samples were similarly examined by Drance et al (1973) showing ACD values at a much *higher level than Eskimos Correspondingly the Lapps* and *Scolts* of Northern Scandinavia agreed with Caucasians (Forsius et al 1970)

The comparison between Eskimos and a rather small sample of *Caucasians living in Greenland* demonstrated a pronounced difference in the ACD levels Table IV also shows the calculated parameters of Tornquist s sample, examined with an earlier but very precise optical method The full agreement between my sample of *Danes* and the 398 *Swedes* is demonstrated The larger Swedish sample is therefore shown for comparison in Figs 2 and 3 At the age of 55 *years the difference between the* ACD levels of Eskimo and Swedish females amounted to 0 38 mm (with the standard error 0 03 mm)

Delmarcelle & Luyckx Bacus (1971) also using the Haag Streit 900 pachymeter method performed a study of ACD in *Belgian* children (1 611 eyes) The sample was selected in three groups according to the refraction. The linear regressions, roughly calculated by me appear in Fig 3 showing that even the Caucasians with hypermetropia had a higher ACD level than Umanaq Eskimo children In continuation of the above mentioned study Weekers Delmarcelle et al (1973) very briefly presented the ACD mean values of 2,395 eyes In the 1 120 adult eyes they found a mean ACD of 3 22 mm in age groups 16–20 followed by 3 10 mm (21–40) 2 84 mm (41–60) and 2 64 mm (> 60) nearly at the same level as Tornquist s sample (Fig 3) The hypermetropic subgroup had the mean values 2 98 2 87 2 61 and 2 58 mm respectively

Thus the last question in the introduction could also be answered The *control* sample of Danes in Greenland did agree with other Caucasians. The ethnic difference in ACD distribution between *Eskimos and* Caucasians received *further support*

With this background a difference in ACD levels between the East and West Greenland Eskimos could have been expected. The elderly Angmagssalik Eskimos form a very pure group due to isolation for several hundred years while the Caucasian admixture in West Greenland is well known and was discernable in all age groups of the districts represented However no general trend towards a Caucasian ACD level appears in the west coast samples of Fig 2 even if the

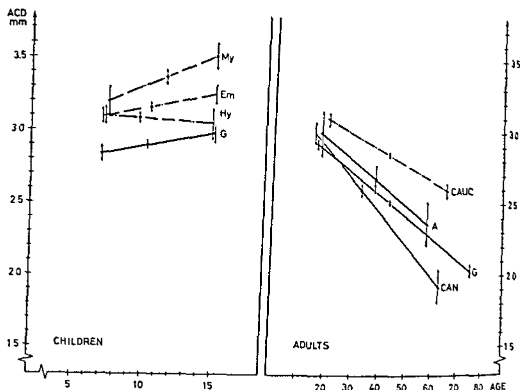


Fig 3

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——— *Eskimos* from G Umanaq *Greenland* A Wainwright *Alaska* and CAN Igloolik *Canada* (Sample sizes are given in Table II)

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The epidemic of myopia observed in adolescent Canadian and Alaskan Eskimos (Young et al 1969 Morgan & Munro 1973 Alsbirk & Forsius 1973) does not seem to exist in Greenland It probably influenced the slope of ACD with age in the Canadian samples

From a clinical point of view the crucial aspect of the ACD survey was the probable association between the low ACD level and the high prevalence of a c g in this population This problem will be dealt with in a later paper However in Fig 2 40 female a c g patients are represented deviating significantly from the cluster of Eskimo population values

In conclusion the present paper shows that compared with Caucasians a shallow anterior chamber is a characteristic of Eskimos from all parts of Greenland and probably of Eskimos from Canada and Alaska also

Acknowledgements

Supported by grants from Fabrikant Einar Willumsens Mindelegat, the Danish Committee for Prevention of Blindness and the Danish Medical Research Council

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most outlying group does belong to the town Sukkertoppen which has had the closest contact with Europeans A further anthropologic discussion will be postponed until later In a later paper the distributions of ACD and corneal diameters will be analysed in relation to a classification of the individual physiognomies as "Eskimo" or "mixed"

Whenever the anterior chambers of Eskimos have been called shallow in the above it is from a Caucasian point of view Comparison of Eskimos with *Mongoloids* would be just as relevant bearing in mind their probable but unproven descent from common ancestors Furthermore, there is some evidence that a.c.g is much more frequent in primary glaucoma series from East Asia than in Caucasian series (Suda et al 1964 Ida Mann 1966 Konyama & Srisupan 1968, Loh 1968) This could imply a lower ACD level in Mongoloids.

So far Mongoloid studies using the Haag Streit pachymeter technique do not seem to have been published Nakajima et al (1968) in a *Japanese* population study of several metric traits of the eye used photographic phakometry The regressions of ACD on age were calculated and included in Table IV for adults A very low level was found also in children, even more pronounced than in Eskimos In another study of *Thai* and *Japanese* adolescents Kimura, Konyama & Nakajima (1969) found the Japanese ACD values ($\bar{y} \pm s_y$) very much higher than in the above material (60 ♂ 3 13 ± 0 31 mm and 30 ♀ 2 81 ± 0 29 mm the Thai values being 0 2–0 3 mm higher) All were emmetropes, aged 12–18 years In this study the measurements were based on slit lamp photographs Thus the results differed considerably in the two studies mentioned probably due to methodological factors As to Mongoloids versus Eskimos and Caucasians we must wait for comparable results

Evidently conspicuous uncertainties immediately appear when the results of studies with *different methods* are compared Systematical discrepancies should always be suspected Correspondingly ultrasound oculometry studies were disregarded in this paper as severe inter equipment differences might be involved especially in measurements of the anterior chamber (Fledelius & Alsbirk 1973) Methodological aspects of optical pachymetry will be considered in a later paper

The *refractive errors* were primarily disregarded as relatively unimportant in the ACD surveys of Greenland Eskimos However a negative correlation is known to exist between refraction and ACD (e.g Stenstrom (1946) $r = -0.34 \pm 0.03$) cf the results for Belgian children in Fig 3 Although a small percentage of Eskimos with myopia exceeding two diopters were known in the Umanaq population the ACD level was considerably lower than for even hypermetropic Caucasians A later paper will consider the distribution of refractive errors in the Umanaq population investigated in 1972

Letter to the Editor

ON THE STILES CRAWFORD PHENOMENON AND RESOLUTION POWER

BY

C. E. T. KRAKAU

In an article published in this Journal (1973) Dr. U. Halldén has treated the possible *effect of the Stiles Crawford* phenomenon on the resolution power of the eye. His intention has been to investigate the possibility of explaining the visual acuity in narrow pupils which is found to be paradoxically high with reference to the diffraction effects. On page 77 H. makes the explicit statement that the Stiles Crawford effect increases the visual acuity by reducing the diffraction. This latter statement will be briefly commented on.

It is possible to change the diffraction pattern of an objective by a procedure called apodization. A coating or mask of varying transmittance over the surface is applied to the pupil (cf. Born & Wolf p. 416). The Stiles Crawford effect is a retinal phenomenon but it is measured at the entrance pupil of the eye. It seems therefore natural to treat the effect as an apodization occurring at the pupil. One might imitate the Stiles Crawford effect by placing a filter of radial symmetry with its highest transmission centrally ($r = 0$) and the lowest at the pupil's margin ($r = R$) according to a function $P(r) = 10^{-r^2}$. Only the amplitude is influenced in this function. If we admit this model to be applicable it is *justified to evaluate the diffraction pattern by calculating the* Fraunhofer integral over the surface of a pupil with apodization. The amplitude v as a function of s is

$$v = 2\pi C k \int_0^R J_0(krs)\, r\, (P(r))\, dr$$

where $k = 2\pi/\lambda$, C is a constant, s the sine of the deflection angle between the diffracted ray and the perpendicularly incident ray, J_0 is the zeroth *order* Bessel function of the first kind.

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ever the same a slight reduction in visual acuity was to be expected from the Stiles Crawford effect

Halldén s discussion is based on Young s diffraction theory which has been given a mathematical formulation by Rubinowicz (cf Born & Wolf p 449) In essence the Young Rubinowicz formulation is obtainable from the Kirchoff integral by transforming the surface integral of the latter into a line integral of the pupillary border There is no difference in principle between the two methods of treatment But the transformation is performed on the assumption that the amplitude is constant over the pupillary surface The Young Rubinowicz formulation is therefore a detour when dealing with the present problem

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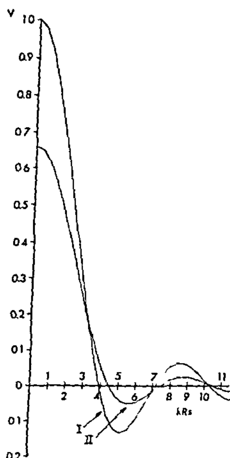


Fig 1
Diffraction pattern (amplitude) of a circular opening I without apodization II with apodization of Stiles Crawford type

Fig 1 shows this calculation for a pupil 8 mm in diameter and a = 0 05 Clearly there is a widening of the diffraction pattern though small even for a dilated pupil as in the example The narrower the pupil the smaller the effect A slight decrease in the resolution power is to be expected for the Stiles Crawford phenomenon not an improvement but as Hallden correctly states in narrow pupils the effect of the Stiles Crawford phenomenon can be ignored There is also a suppression of secondary maxima by an apodization of the Stiles Crawford type a fact which may favourably influence the perception of a faint object (like a star) adjacent to a bright one but this is not an acuity improvement according to accepted criteria

A very similar problem was treated by Metcalf (1965) though this author used an experimentally determined spread function instead of the theoretical pattern of a circular aperture The results obtained by this author were how

Bernard M J Orthoptie Pratique, Doin Paris 1973 192 pages 114 figures In French Price FFr 48

The author of this book who has had many years of experience as orthoptist and teacher has wanted to help future orthoptists by giving them a practical introduction in the use of the instruments and examination techniques which are the basis of orthoptic examination.

It also gives an adequate description of how to treat amblyopia. No other treatment methods are mentioned

The text is clear and concise and is supplemented with a series of good illustrations

For orthoptists this book can serve as a valuable precursor to more comprehensive textbooks

K Norskov

Ciba Foundation Symposium 19 (new series) The Human Lens – in Relation to Cataract Associated Scientific Publischers Amsterdam 1973 324 pages Price DFl 43.50

This book comprises contributions and related discussions from some of the most outstanding lens investigators

Clinical morphological chemical physiological and immunological aspects of the subject in question are presented One of the main problems in cataract research – the absence of a generally accepted classification of cataract – is reflected in the various contributions While Friedburg related his data to the following types of cataract subcapsular deep cortical nuclear and totally opaque lenses other investigators distinguished between cataractous lenses according to their contents of pigment and others applied microdensitometric measurements No classification was agreed upon at this conference.

Among the interesting information is Maraini s statement that the total amount of lens protein decreases and the water content increases as well as the sodium content – even in early stages – in cortical opacification On the contrary in nuclear cataractous lenses such changes were not observed Instead proteins were found to be insolubilized, which was not observed in cortical cataract Philipson & Fagerholm using microradiograms showed that cortical cataract might be due to changes in permeability of the lens fibers Kinoshita suggested that the glutathione of the lens protected thiol groups of the lens proteins from being oxidized – especially in the presence of Ca^{+} – which might cause polymerization and aggregation of lens proteins and possibly cause nuclear cataract probably not cortical cataract

One aspect seems to have been partly neglected namely the energy metabolism of the ageing and cataractous lens. Only Friedburg s outstanding investigations in the field of enzyme activity patterns in various forms of cataract deals with this subject. The book would have been further enriched in this respect by the participation of certain other German investigators as well

Conclusively it may be said that this book reflects the present knowledge on most aspects in senile cataractogenesis and lens ageing It is a valuable book for anyone to whom cataract is a puzzle as well as an everyday clinical problem. Although some processes in cataract development are known, the trigger mechanisms in senile cataract remain a mystery

A Bruun Laursen

Lawton Smith J & Glaser Joel (ed) Neuro ophthalmology Vol 7 Symposium of the University of Miami and the Bascom Palmer Eye Institute Mosby Saint Louis, 1973 xv + 146 pages 109 illustrations Price $ 23 65

As usual most excellent papers are presented 1 Three major sins in neuro ophthalmology (J Lawton Smith) 2 Optic nerve hypoplasia in aniridia (Paul R Layman) 3 Optic disc edema revisited (Martin Lubrow) 4 Toxic and deficiency optic neuropathies (Simmons Lessell) 5 Ischemic optic neuropathy (Ronald M Burde) 6 Developmental tumors about the optic chiasm (Larry K Page) 7 Transsphenoidal encephalocele associated with colobomas of the optic discs and hypopituitary dwarfism (Leopold J Streletz and Norman J Schatz) 8 Ophthalmic aspects of the battered child syndrome (Brain Harcourt) 9 Improving visual acuity in congenital nystagmus (L F Dell Osso) 10 Techniques of microvascular surgery (R M Peardon Donaghy) 11 Abstracts of papers and discussions during the free paper presentation session (Joel S Glaser)

P Brændstrup

Leopold Irving H (ed) Symposium on Ocular Therapy Vol 6 Mosby Saint Louis, 1973 ix + 112 pages 14 illustrations Price $ 17 50

12 most excellent papers are presented

1 Diphenylhydantoin and its use in optic nerve disease (Bernard Becker and Steven M Podos) 2 Ocular changes in drug abusers (Robert P Burns and Arthur Steele) 3 Cardioactive glycosides in ophthalmology (Wayne F March Seymor B Goren and David Shoch) 4 Antiglaucoma drugs problems with carbonic anhydrase inhibitors (W Morton Grant) 5 Prophylaxis of ophthalmia neonatorum (John E Harris) 6 Acupuncture and the consideration of placebo effects (William H Havener) 7 Herpes therapy (Herbert E Kaufman) 8 Therapy of chronic adenoviral infection (Peter R Laibson) 9 Systemic antifungal chemotherapy in the treatment of intraocular fungal infections (Theodore W Lieberman) 10 Current status of radionuclides in ophthalmology (Frank W Newell) 11 The effect of pilocarpine Ocusert on ocular pressure (Mansour F Armaly and Kosaraju R Rao) 12 Drug interactions (Irving H Leopold and Bruce Gordon)

P Brændstrup

Hurtt et al Comprehensive Review of Orthoptics and Ocular Motility C V Mosby St Louis 1972 253 pages 52 illustrations Price

This book is the joint work of two orthoptists and an ophthalmologist The authors have tried to cover the American Orthoptic Council s requirements in examining orthoptists They have chosen the question and answer method of presentation which may or may not have been advisible All the same they have managed to produce readable and informative material on the theory of orthoptics diagnosis and treatment in relation to surgical procedures First and foremost the book is of interest where orthoptists train and work and also for ophthalmologists who are interested in the combination of orthoptic and surgical treatment

K Norskov

International Symposium of Paediatric Ophthalmology Parma October 4 5 and 6 1974

Preliminary programme

1) Round Table (October 4) The functional examinations of the visual functions in the newborn and in childhood Moderators H Burian M D (Chapel Hill) C. Lombroso M D (Boston) Subjects The pupillary reflex and pupillography in the newborn (F Trimarchi Pavia) Electroretinography (M Cordella Parma) Visual evoked potentials (L Maffei Pisa) Optokinetic nystagmus and visual acuity The following communications have been submitted Ecography and electroretinography in retrolental fibroplasia. Anisovision Anaesthesiological problems in examination of the newborn Electroretinography in malabsorption and galactosemic diseases

2) Round Table (October 5) Diagnostic pathogenetic and therapeutic problems in ophthalmological diseases of the newborn Moderators J François M D (Gand) E Sartori M D (Padova) Subjects Malformations and embryopathies J François (Gand) Problems in perinatal diagnosis B Salvadori (Parma) Prematurity and the eye Cranio facial dysostosis R Brizzi F Carta (Parma) Leucocoria B Boles Carenini (Cagliari) Fundus angiography in the premature and in the newborn P Amalric (Albi) The following communications have been submitted Surgical problems in rubella cataract and in soft cataract Pathogenic problems of corneal dystrophies Rare congenital ocular diseases Retinoblastoma diagnosis and therapy

3) Round Table (October 6) Diagnostic pathogenetic and therapeutic problems in phthalmological diseases of childhood Moderators J Babel M D (Genève) P Durand M D (Genova) Subjects Genetic diseases Neuro epithelial degenerations Degenerations of the optic pathways M Maione (Parma) Inborn errors of metabolism Deficiency and malabsorption syndromes Uveal inflammations The following communications ha e been submitted Ophthalmoscopic pictures in the child after haemodyalisis Ectopia lentis Ocular diseases and enzyme deficiencies Vitamin A transport Retinal detachment in ch ldhood Glycolipidosis The time table of the meetings will be the following 09–1?.30 Round Tables and closed discussion. 12,30–14,30 lunch interval 14.30–17 communications and open discussion on the Round Table subjects Participation in the Symposium is free Members will receive in advance the texts discussed in the Round Tables. A fee is foreseen for those wishing to participate in the social programme

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The Third Symposium on the Structure of the Eye

Foll wing the Tenth Internat nal Congress of Anatomists in Tokyo August 25–30 1? 5 the Third Symp sium on the Structure of the Eye will be held at Hotel Mt Fuji from August 31 to September 3 19 5 Those interested in participating please contact either Profe or S M shima, Dept of Ophthalmology or Professor E. Yamada, Dept of Anat my Faculty of Medicine, University of Tokyo Hongo Tokyo 113 Japan.

Schul e E Die Opthalmo Elektromyographia Georg Theme Leipzig 19/? 66 illustrations 11 tables Price DM 55 20

This monograph gives a thorough review of ophthalmo electromyography The introduction describes its fields of activity The next section describes the development of ophthalmo electromyography the construction of the outer eye muscles the electrophysiological basis for electromyography and the electrophysiological characteristics of the eye muscles A long detailed description follows giving earthing technique with electromyography the different types of electrodes and most of the recording apparatuses presently on the market The following chapters review analysis of the electromyogram coordination of the electromyographical findings in eye motility and the different methods for noting the position of the eye in conjunction with electromyographically recordings

The electromyographical results must be seen in relation to the pathophysiological changes in the muscle It is easy enough to take a biopsy from a skeletal muscle but eye muscles are more difficult and the author takes up these problems in great detail

The next section describes electromyography of different types of eye movement innervation coordination and the electromyographical analysis of disturbed motility – the different forms for neurogenic paralyses denervation and reinnervation A description is given of the clinical picture in the rare cases of ocular myasthenia levis and myasthenia gravis and examples of electromyographical recordings of these diseases are given In the author s opinion a diagnosis of ocular myasthenia can only be made when electromyography is combined with a tension test As it can be difficult to make a differential diagnosis of the different forms of ocular myopathy a thorough description of the clinical histological aspects of these diseases is also included The author finds that in ocular myopathy there is an interference pattern in the electromyographical recording The potential amplitude of the motor element is reduced and potential duration is shown with a broad variation of 0 6–1 2 msec This is also seen in ocular myopathy and to some degree in ocular myositis With paralysis of the eye movement the innervation distribution between agonists and antagonists is disturbed whilst the motor elements in the individual muscles are normal Examples of this are shown during electromyographical recordings from several eye muscles

The book thoroughly reviews the principles of electromyographical recording and indicates where electromyography can be of help in diagnosing disturbances of the outer eye musculature the value of an electromyographical examination is in being able to show deviations from the norm a more thorough description of the parameters of the motor element s potentials would have been desirable However as a whole it is an excellent and well written book that deserves recommendation It also includes a very comprehensive reference list

Svend Faurschou Jensen

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THREE CASES OF NON-INFLAMMATORY ISCHEMIC OPTIC NEUROPATHY OCCURRING IN THE SAME FAMILY

BY

L. BERGGREN, W. THORBURN and H. FODSTAD

In the family presented here, three out of seven siblings are affected, the onset of symptoms having occurred between 53 and 59 years of age. The family relationship was at first unknown. The parents are believed to have been eye healthy. Two of the cases are bilaterally involved and were investigated for suspected brain tumor. The neuroradiological examinations *were negative. The visual symptoms, i.e. loss of visual acuity, visual field* defects and blurring of the optic discs were in agreement with a diagnosis of ischemic optic neuropathy. In the latest case discovered, the affliction is thus far unilateral, and vascular changes in the retina corresponding to the visual field defect could be established by fundus photography and fluo*rescein angiography.*

Key words: ischemic optic neuropathy – segmental infarction of the optic nerve – vascular pseudopapillitis – visual field defects

Ischemic neuropathy of the optic nerve (vascular pseudopapillitis or segmental infarction of the optic nerve) can at times be difficult to diagnose on the basis of clinical signs. Patients presenting blurred discs and visual field defects are

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Neuro Ophthalmology Course

Bascom Palmer Eye Institute, Department of Ophthalmology University of Miami School of Medicine January 6–9 1975 Doral Beach Hotel Miami Beach Florida. Two courses will be offered this year The *BASIC* course in Clinical Neuro Ophthalmology will be January 6–7 and will include the neuro ophthalmological examination, pupil optic nerve visual fields cerebral disease, III IV VI diseases nystagmus, and the orbit There will be an *ADVANCED* course January 8–9 and this will emphasize diseases of the ocular muscles and unusual forms of oculocerebrovascular disease. This faculty includes Dr Douglas Anderson Dr Robert Daroff Dr R M Peardon Donaghy Dr J Donald M Gass Dr Joel Glaser Dr Simmons Lessell Dr John A McCrary Dr Edward W D Norton Dr O M Reinmuth Dr Norman Schatz Dr J Lawton Smith Dr John O Susac. Registration fee is $ 125 for practitioners and a special rate of $ 50 for residents for each course, or $ 250 for practitioners and $ 100 for residents for both courses upon application from their Department Head Interesting patients and clinical material will be presented for discussion at both courses Check (U S dollars only) should be made payable to Neuro Ophthalmology Course and mailed to Bascom Palmer Eye Institute P O Box 520875 Biscayne Annex Miami Florida 33152 Special reduced rates have been arranged with the Doral Beach Hotel 4833 Collins Avenue. Miami Beach Florida 33140 USA

The Second International Symposium of Eye Surgery

will be held in Bologna from May 25th to 29th 1975 The subjects to be treated are ocular orbitary prosthesis implants strabismus and nystagmus detachment of the retina surgery of the vitreous Simultaneous translations will be available in English French Italian and German Registration Fee 150 dollars For particulars please contact Prof G Cristini II Simposio Internazionale di Chirurgia Oculare Clinica Oculistica Università Via Massarenti 9 Bologna Italy

Department of Ophthalmology
(Head Prof E Linner) and Department of Neurosurgery (Head C A Thulin MD)
University of Umea Umea Sweden

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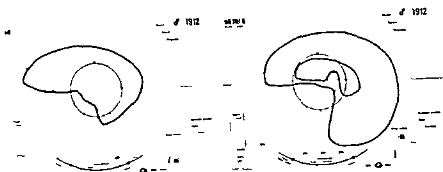


Fig 1
Case 1 Right eye affected first followed one year later by symptoms from the left eye Visual fields show inferior left defects crossing the midline

pressure was 160/100 Sedimentation rate X rays of the skull optic foramina and sella turcica encephalography EEG carotid arteriographies on both sides brain scintigram and liquor examination were normal Treatment with corticosteroids (prednisolon 0 mg daily for 1 week) was without any effect

Case 2

A woman born in 1913 who in her own opinion had previously had normal visual function At 54 years of age she suffered from an acute loss of vision in the right eye Ophthalmological examination 1 month later revealed a pale atrophic disc in the right eye This eye was amaurotic In the left eye the corrected vision and visual fields were normal There was no blurring or protrusion of the left disc Three years later at 57 years of age the vision diminished in the left eye Ophthalmological examination 3 weeks after onset established a corrected vision of 0 2 (?0/80) in the left eye The left disc was choked with a protrusion of 3 D The fundus examination also revealed venous stasis and a retinal hemorrhage and there were marked variations in caliber of the retinal arteries The caliber variations in the right eye were less pronounced The right disc was pale and atrophic as in the previous examination. Visual fields suggested a lower nasal defect of the left eye. A fundus examination 1 month later showed the left disc to be blurred but the protrusion had decreased to 1 D The hemorrhage had disappeared The variation in caliber of the arteries was unchanged The visual field defect in the left eye had by then progressed to an incomplete nasal defect (Fig 2)

General physical and neurological examinations were within normal limits The blood pressure was 180/100 Sedimentation rate X rays of the skull optic foramina and sella turcica, carotid arteriographies on both sides encephalography EEG and liquor examination were normal Treatment with ACTH (60 to 15 IE daily during 2 weeks) was without effect

often suspected of having a brain tumor and given thorough neurological examinations while those with arcuate scotoma have sometimes been diagnosed as having glaucoma However in most cases the clinical manifestations are quite typical Investigation of the visual fields is of particular importance as it makes early diagnosis possible resulting in a reduction of the neuroradiological studies (François et al 1957 1962 Piper & Unger 1957 1963 Enoksson 1965 Miller & Smith 1966 Walsh 1969 and Duke Elder 1971) To the best of our knowledge no report of cases of ischemic optic neuropathy occurring in the same family has been presented

Case Reports

General In a family living in a sparsely populated area in the most northern part of Sweden five siblings born 1895–1903 were considered eye healthy After the death of the mother the father remarried Three out of seven brothers and sisters from this second marriage born 1905–1920 have been affected a brother born in 1912 a sister born in 1913 and the youngest sister born in 1920 There is no information of similar symptoms in the father his second wife or the grandparents on either side The onset of symptoms in the three cases have been between 53 and 59 years of age The eldest brother and sister have a bilateral involvement and the youngest sister has hitherto symptoms from one eye only

Case 1

A man born in 1912 who in his own opinion had previously had normal visual function At 58 years of age he noticed a decrease in vision in the right eye Ophthalmological examination one month later established a corrected vision of 0 / (20/30) in the right eye and 1 0 (20/20) in the left eye There was blurring of the disc margins and a protrusion of 1 5 D of both discs The retinal arteries were moderately arteriosclerotic The veins were considered normal and no retinal hemorrhages were seen Visual fields showed a normal left field and a lower nasal defect crossing the midline in the right field One year later at 59 years of age the vision in the left eye suddenly diminished Corrected vision was still 0 7 (20/30) in the right eye In the left eye vision was reduced to ability to count fingers at 1 meter (4/200) The margins of the discs were now distinct and no protrusion was found Visual field examination revealed a lower temporal defect in the left field and the defect in the right field reported above The findings 5 months later were almost identical to these (Fig 1)

General physical and neurological examinations were within normal limits The blood



Fig 4

Case 3 Photograph of the left fundus Note narrowing of upper retinal artery at the disc and pathological arteriovenous crossings in the upper part





Figs 5a and b

Case 3 Fluorescein angiography of the left eye a) Early arteriovenous phase b) Late venous phase. The pathological findings seen in Fig 4 are also seen here



Fig 2

Case 2 Right eye affected first followed 3 years later by symptoms from the left eye. The right eye is amaurotic and the left visual field shows an incomplete nasal defect.

Case 3

A woman born in 1920 (the youngest family member) had a sudden decrease of vision in her previously healthy left eye at 53 years of age The corrected vision was 1.0 (20/20) in the right eye and 0.2 (20/80) in the left eye The right visual field was normal while the entire lower part of the left visual field was defect (Fig 3) The optic disc and retinal vessels in the right fundus were normal The left disc was initially choked, with a protrusion of 3 D The disc findings were completely normalized within 2 weeks. In the left fundus it was further noticed that the upper retinal artery was narrowed in its most central course In this region pathological arteriovenous crossings were also

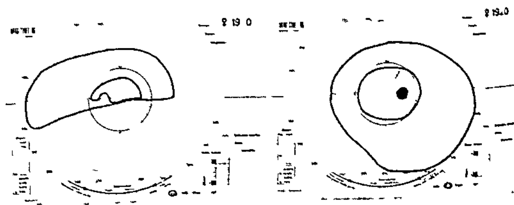


Fig 3

Case 3 Left eye affected Right eye to date (1973) normal Visual fields show a normal right field and an inferior altitudinal defect in the left field

may be misinterpreted as a sign of raised intraocular pressure Careful exami nation of the optic discs retinal vessels and particularly the visual fields will provide the correct diagnosis The typical field defects such as sector defects arcuate scotoma and altitudinal defects cross the vertical midline. The symp toms of the cases from the same family reported here agree well with a diagnosis of ischemic optic neuropathy It is unlikely that the symptoms should have occurred by chance and it seems plausible to assume a common defect in the vascular nutrition of the optic nerves The examinations including carotid angiographies have however not been able to reveal the exact nature of the underlying disease

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found (Fig 4) Other vessels in the left fundus were considered normal There were no hemorrhages or exudates Fluorescein angiography was used as a further aid to visualize the pathological retinal vessels No fluorescein leakage was found (Figs 5a and b)

General physical and neurological examinations were within normal limits The blood pressure was 140 90 Sedimentation rate X ray of the skull and carotid arteriographies on both sides were normal ACTH injections for a week were without effect There were no changes in the appearance of the retinal vessels or the extent of the visual *field defect 1 month later*

Discussion

As no neurological cause for the visual failure in the two bilateral cases could be found the ages of the patients and the retinal vascular findings indicate that *the visual symptoms may have been due to vascular disturbances in the optic* nerve The third case which is unilateral a more thorough ophthalmological examination showed marked pathological changes in the upper branch of the central artery The arterial changes corresponded well to a defect of the lower visual field All three cases had normal erythrocyte sedimentation rates

The occurrence of obliteration of the nutrient vessels of the optic nerve is well documented Vascular occlusion due to inflammatory processes such as giant cell arteritis are not uncommon They are combined with a highly increased erythrocyte sedimentation rate Vascular occlusion due to degenerative changes are seen less often

The clinical symptoms of non inflammatory ischemic optic neuropathy in clude changes in the optic disc visual field defects and loss of visual acuity The age of onset is usually between 45–70 years of age The condition may be unilateral but the second eye is often affected at a later date Pain is absent The erythrocyte sedimentation rate is normal The visual manifestations may develop rapidly or slowly and the loss of vision is permanent The visual defect varies from complete blindness to visual field defects particularly lower altitu dinal defects and arcuate defects or less frequently central scotoma The optic disc is often blurred and protruding and is sometimes seen in combination with retinal hemorrhages and exudates Pathological retinal vessels are often seen Sometimes a pale ischemic optic atrophy may be found The optic disc findings may be misinterpreted as a sign of raised intracranial pressure A choked disc on one side and a pale disc from a previous attack on the other side is some times misinterpreted as the Foster Kennedy syndrome An arcuate visual defect

In later years a great many contributions have been published in ophthalmolo gical reviews from many countries especially Scandinavia about an eye disease called senile exfoliation fibrillopathy or pseudoexfoliation among other names but degenerative exfoliation seems to be the most appropriate These studies have often shown varying results and in spite of electron microscopical and biochemical experiments the origins of the disease have not been found In my practice I have had cases of senile exfoliation especially among patients with open angle glaucoma It struck me as being a rather rare disease but on further examination it turned out to be otherwise Icelandic ophthalmologists have not made any extensive studies on this disease with or without glaucoma *although* we probably treat comparatively more cases of glaucoma than our Scandinavian colleagues

It was not until the Human Ecologic Research team under Prof H Forsius of Uleå Finland began their studies on senile exfoliation in the summer of 1972 that I began systematically to examine older people who came to my consulting rooms with symptoms of this disease

Many Scandinavian colleagues e g H Forsius and A Tarkkanen (Finland) E Horven T J Bertelsen Th L Thomassen H P Petersen and H Aasved (Norway) and Ladekarl (Denmark) have made remarkable studies on the fre quency of senile exfoliation (fibrillopathy) in their countries and elsewhere These and other studies have shown that senile exfoliation mainly occurs in people of 60 and over it is similar among men and women white men and coloured in warm and cold countries in short all over the world

The different frequencies for this disease in various countries are very likely the result of different research methods and materials as pointed out by H Aasved but in some instances chance might be responsible as when Prof W Leydhecker did not find a single case of exfoliation *in 500 glaucoma pa* tients in spite of extensive research Prof Th L Thomassen found in England [illegible]% exfoliation among glaucoma patients whereas various Norwegian resear chers have found 0–80% Thus there is considerable difference in the results

The object of this study was to examine the frequency of senile exfoliation among Icelanders especially among open angle glaucoma patients and to find whether our cold and unstable climate heredity and other factors may be influencing the frequency thereby giving a different result *from our neighbour* ing Scandinavian countries

It is obvious that in such a small *material one* can only get a slight idea of the real situation My examination covered 1 280 patients of whom 214 had open angle glaucoma ages 50–100 years In such a study it is necessary to distinguish between patients with and without glaucoma to know what stage the disease has reached how long it has lasted and the age of the patients etc

Reykjavik Iceland

THE FREQUENCY OF SENILE EXFOLIATION IN ICELAND

Fibrillopathy or pseudoexfoliation

BY

KRISTJAN SVEINSSON

A total of 545 men and 521 women of 50 years and older were examined for senile exfoliation without discernible glaucoma. Here as elsewhere exfoliation mainly occurs after the age of 60. In male patients exfoliation without glaucoma was found to be more frequent than in women (2.9 % – 1.7 %). In the material 214 patients with open angle glaucoma were examined, of whom 147 were men (average 76 years) and 67 women (average 77 years). There were 84 men (57 %) and 31 women (46 %) with senile exfoliation.
When looking for the causes or effects of senile exfoliation in glaucoma afflicted eyes I am led to believe that in most cases it is primarily caused by glaucoma simplex, the exfoliation being the result of a long standing increase of tension with ensuing degeneration. More specifically, that in the ciliary and iris epithelial layers mucopolysaccharide or other unknown substances are developed and occur as exfoliation materials in the various tissues of the eye.

Key words: pseudoexfoliation of the lens capsule – fibrillopathia epitheliocapsularis – glaucoma simplex – Iceland

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As shown in Tables I and II senile exfoliation increases with age here as elsewhere It might be accidental that the disease seldom occurs in either sex until after 60 Of these 1 066 cases 25 had exfoliation (2 3 %) the percentage for men was considerably higher than women 2 9 % and 1 7 % respectively
Exfoliation in these cases was found in both eyes centrally and peripherally

The frequency of senile exfoliation (fibrillopathy) in patients with open-angle glaucoma

It is well known that the glaucoma disease expresses itself in various ways in different countries in some countries closed angle glaucoma is more common in others open angle glaucoma In my private glaucoma material that consists of over 2 000 patients only 2 % and less of the cases have closed angle glaucoma The slow glaucoma simplex was formerly a serious menace in Iceland while there were few ophthalmologists in the country I collected and studied information from other ophthalmologists on all blind persons in the whole country most of them had been examined in 1940 by specialists It was then revealed that 71 1 % of blindness in Iceland was caused by glaucoma In my material males are about twice as frequent as females 66 % and 34 % respectively

During the past two years 214 patients with open angle glaucoma were examined for senile exfoliation There were 147 men (68 7 %) and 67 women (31 3 %)

The intra ocular pressure was measured by means of standardized Schiotz weight tonometer using the calibration of 1955 The anaesthetic used was 0 4 % Novesin I examined 150 of the patients in my consulting rooms with a slit lamp in a darkened room but 64 patients were examined in the provinces where a slit lamp was not available There I used Keeler s pantoscope slit with a strong light in a darkened room It was often necessary to dilate the pupil for this I used mydriacyl and neosynephrin drops but many of the patients had miotic pupils and examination conditions did not permit dilatation

In this material patients with closed angle and absolute glaucoma are not included only patients with open angle glaucoma representing different degrees of severity

By looking at Tables III and IV we see that out of 147 men average age 76 years with open angle glaucoma senile exfoliation is found in 84 or 57 % and of 67 women with the same disease average age 77 years senile exfoliation is found in 31 patients or 46 % total of both sexes is 53 5 % Eighty three had been operated on for glaucoma and senile exfoliation was found in 50 % of these In 30 % of these patients senile exfoliation was found in only one eye

Most of my patients were Icelanders who have lived in Iceland most or all of their lives, either in the country or towns

Patients without glaucoma

The material consisted of 1 066 men and women of 50 years or older (545 men 521 women) On examination and tension test no traces of glaucoma are found In many cases the pupil was dilated by mydriacyl drops

Tables I and II show the incidence of senile exfoliation in men and women without glaucoma

Table I

Incidence of senile exfoliation in men without glaucoma

Age	No investigated	No with senile exfoliation	%
50–60	162	0	0
60–70	171	1	0.58
70–80	154	8	5.2
80–90	55	7	12.7
90–100	3	0	0
Total	545	16	2.9 %

Table II

Incidence of senile exfoliation in women without glaucoma

Age	No investigated	No with senile exfoliation	%
50–60	178	0	0
60–70	156	0	0
70–80	129	6	4.6
80–90	53	3	5.6
90–100	5	0	0
Total	521	9	1.7 %

In half of the cases I found a central exfoliation disc or ring in the front side of the lens but in all patients where it was possible to examine the peripheral side part of the lens an exfoliation girdle was found round the periphery of the anterior lens capsule with flakes on the pupillary margin In these 214 glaucoma patients representing different degrees of severity most of them had optic nerve damage the incidence of exfoliation glaucoma in our country seems to be pretty much the same as elsewhere. Henry Aasved finds for example in out patients an incidence of fibrillopathy of 42 4 % in glaucomatous patients with optic nerve damage in my study the incidence is 51 5 % or slightly higher Therefore it seems as though our cold and stormy climate does not have much influence on the frequency

As to heredity is is difficult to gauge its influence from this scanty material But as glaucoma is obviously a hereditary disease one might expect the exfoliation characteristics to be in some degree hereditary Of the above 214 patients seven pairs were brothers and sisters of whom four pairs had exfoliation characteristics one pair was free and two pairs had mixed The strongest affliction of senile exfoliation was observed in a 70 year old patient without glaucoma whose mother had suffered from glaucoma simplex in both eyes This might be a case of what A Tarkkanen and his colleagues tentatively call autosomal dominant gene with incomplete penetration and variable expressivity

Discussion

Capsular glaucoma is still considered by many ophthalmologists to be secondary glaucoma but others regard it as glaucoma simplex I am also of the opinion that it is simple glaucoma an abiotrophic senile phenomenon rarely encountered below the age of 60

Glaucoma simplex begins in most cases long before suspected and has therefore existed long before the standard symptoms appear The varying heavy but long standing increased pressure in the eye weakens the whole organism not only the neuroepithelium and *nervus opticus* but also by causing alterations in the lens refraction and degeneration in the ciliary and iris epithelium in the fine iris veins it entails a stronger hyalinization in the trabecular and Schlemm s canal One might imagine that mucopolysaccharide or other unknown substances cause a fallout of exfoliation material in the various afflicted tissues of the anterior eye which then slowly cause peculiar pathological alterations in the trabecular and Schlemm s canal increasing the gravity of the disease.

Table III

Open angle glaucoma

Males with and without senile exfoliation incidence

Age	No of patients with senile exfoliation	%	No of patients without senile exfoliation	%
50–60	1	1	6	9.5
60–70	12	14.3	19	30.1
70–80	51	60.8	27	43.0
80–90	20	23.9	11	17.4
90–100	0	0	0	0
Total	84	100 %	63	100 %

Average 57 % Average 43 %

Average age of men 76 years

Table IV

Open angle glaucoma

Females with and without senile exfoliation incidence

Age	No of patients with senile exfoliation	%	No of patients without senile exfoliation	%
50–60	0	0	3	8.4
60–70	3	9.7	10	27.8
70–80	13	42.0	14	38.8
80–90	14	45.1	9	25.0
90–100	1	3.2	0	0
Total	31	100 %	36	100 %

Average 46 % Average 54 %

Average age of women 77 years

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In cases where exfoliation occurs without glaucoma the causes might be that the pathological alterations of exfoliation affect the trabecular tissue and Schlemm s canal to some degree and after a varying length of time We have observed *in many cases of exfoliation without glaucoma that the glaucoma* eventually appears *vi.* it takes a varying length of time for it to develop and spread in the trabecular tissue and Schlemm s canal These typical pathological tissue alterations take place after the exfoliation and therefore many patients have a clear exfoliation outflow system for many years

But why does not exfoliation occur in all eyes with open angle glaucoma? It is difficult to find an answer The eyes are to a different degree resistant to increased tension Possibly the duration of the affliction and the degree of tension are relevant factors

We often observe that glaucoma and especially cataracts only afflict one eye *which shows that degenerative alterations in the human body* affect the organs to a different degree

Cataracts In my material exfoliation with cataracts does not seem more common than in the population as a whole

Maculadegeneration I have seen elderly patients suffering from macula degeneration with exfoliation but there are many more people who in spite of advanced age are free of this disease

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EFFECT OF SOFT LENSES ON CORNEAL SENSITIVITY

BY

MICHEL MILLODOT

Corneal sensitivity was measured prior to inserting hydrophilic lenses for 8 hours just after removal and again 15 min and 1 hour later. It was found that the sensitivity was not significantly different immediately after 8 hours of wear as compared to its initial value in the morning. However after the lens had been removed for 1 hour corneal sensitivity had diminished to a significant level indicating that soft lenses affected the sensitivity. This is understandable because corneal sensitivity normally increases throughout the day. It is felt that the reduction of corneal sensitivity induced by soft lenses may be due to some corneal oedema which might develop because the lenses hinder the evaporation of tears.

Key words: cornea – sensitivity – oedema – soft contact lenses

Conventional contact lenses are known to affect the sensitivity of the cornea and it has been documented that corneal sensitivity diminishes after the continuous wear of these lenses (see review by Millodot 1971). This reduction of corneal sensitivity may be attributed principally to the oedema induced by the wear of contact lenses. Similarly in the case of soft lenses there is evidence of corneal oedema. The work of Fatt & St. Helen (1971) and Morrison & Edelhauser (1972) showed that the oxygen transmission was sufficient to meet the basic need of the cornea. On the other hand Hill & Augsburger (1971) report that the oxygen tension under both types of contact lenses was inter

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In cases where exfoliation occurs without glaucoma the causes might be that the pathological alterations of exfoliation affect the trabecular tissue and Schlemm's canal *to some degree and after a varying length of time* We have observed in many cases of exfoliation without glaucoma that the glaucoma eventually appears *viz* it takes a varying length of time for it to develop and spread in the trabecular tissue and Schlemm's canal These typical pathological tissue alterations take place after the exfoliation and therefore many patients have a clear exfoliation outflow system for many years

But why does not exfoliation occur in all eyes with open angle glaucoma? It *is difficult to find an answer The eyes are to a different degree* resistant to increased tension Possibly the duration of the affliction and the degree of tension are relevant factors

We often observe that glaucoma and especially cataracts only afflict one eye which shows that degenerative alterations in the human body affect the organs to a different degree

Cataracts In my material exfoliation with cataracts does not seem more common than in the population as a whole

Maculadegeneration I have seen elderly patients suffering from macula degeneration with exfoliation but there are many *more people who* in spite of advanced age are free of this disease

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lenses and a measurement of his corneal touch threshold was made subjectively (Millodot 1973) The measurements began with stimulation of the cornea at the lowest pressure and continued *in an ascending fashion* At each pre determined length of the nylon monofilament 4 to 6 contacts were made with at least one blank to test subject reliability The subject indicated when he felt the probe by pressing a bell From these readings the *touch threshold* was defined as the length of the monofilament for which the subject responded for 50 % of the number of stimulations This length was *converted into* pressure using the calibration curve of the instrument relating length and pressure previously established (Millodot 1969) The subjects were then asked to report 8 hours later They removed their lenses in the laboratory and the testing of their corneal touch threshold was carried out within a minute of removal The subjects stayed in the laboratory for 1 hour and further testing was repeated 15 min and 1 hour after the removal of their soft lenses

Results

The results are given in Fig 1 Each data point of the *dotted line represents* the mean of 12 subjects The mean corneal touch threshold (that is the inverse of the sensitivity) just prior to inserting the soft lens at 9 a m was 23 19 mg sq/mm After 8 hours of continuous wear the mean corneal touch threshold was found to be 28 17 *mg/sq/mm approximately 1 min after removal* This *mean value is not significantly different* from the preceding measurement ($t - 0\ 7$, $P > 0\ 1$) However it cannot be inferred from this result that soft lenses do not affect corneal sensitivity as this can only be assumed to be so if the sensitivity had remained the same after the lenses were removed Yet after 15 min the mean corneal touch threshold decreased to 23 42 mg/sq/mm and after 1 *hour the mean corneal touch threshold* was found to be 20 21 mg sq mm which is lower than prior to inserting the lenses in the morning or just after removal The difference between the mean corneal sensitivity value after the lenses have been removed for 1 hour and that obtained just after removal is statistically significant ($t = 1\ 9$ $P = 0\ 05$) Thus the hypothesis that soft lenses do have an *effect on corneal sensitivity* must be accepted All subjects but one displayed a decrease of corneal sensitivity just after removal of the lenses In one instance this reduction was 100 % and the cornea of this subject was found in a slit lamp examination to have a central oedema and the eye was more myopic by about 1 5 diopters In all subjects though corneal sensitivity increased within the hour after *removal of the soft lenses.*

fered with to about the same extent Various investigators have demonstrated that the wearing of soft lenses scarcely alters the curvature of the cornea (Bonnet & El Hage 1968 Knoll Harrington & Williams 1970 Dyer 1970 Larke 1972) But little or no change in curvature does not necessarily mean that the cornea is physically unaffected as it could nevertheless increase its thickness uniformly without changing the corneal curvature. This is indeed what Bailey & Carney (1973) have shown after measuring both corneal thickness and curvature after wearing soft lenses

The corneal sensitivity after the wear of soft lenses has been assessed by Knoll & Williams (1970) and Larke & Sabell (1971) and both found it to be reduced but not significantly The measurements were made after 6 hours of wear in Knoll & Williams s study and after a length of wear which is unspecified in Larke & Sabell s report In view of Baily & Carney s study (1973) on the corneal oedema induced by soft lenses and cursory observations by several clinicians it seems hardly plausible that corneal sensitivity would remain totally unaffected by the wear of soft lenses and it is the purpose of this study to further evaluate this effect taking into account the fact that corneal sensitivity normally varies throughout the day (Millodot 1972)

Material and Method

The Cochet Bonnet Aesthesiometer (1960) based on the instrument devised by Boberg Ans (1955) was used to stimulate the cornea Two models of the instrument with nylon monofilaments of 0 12 mm and 0 08 mm diameter were used to produce pressures between 11 mg to 20 mg/0 0113 mm² and 2 mg to 90 mg/0 005 mm respectively The aesthesiometer was mounted in a holder allowing movement in x y and z axes so that reliability in stimulation of a corneal point, steady speed of application (Boberg Ans 1956) and a perpendicular corneal contact was achieved A corneal point near the limbus in the 6 o clock position was stimulated and the slightest bend of the nylon wire visible through a X4 3 magnifier was defined as corneal contact All measurements were taken when the humidity in the room was between 20 and 40 % because the nylon monofilament is affected by humidity (Millodot & Larson 1967)

Twelve subjects between 21 and 27 years of age participated in this experiment They were free of ocular pathology and were wearing soft hydrophilic lenses for periods ranging from 1 day to 3 months

Each subject reported in the morning around 9 a m prior to inserting his

(that is a reduction of threshold) throughout the day The measurements obtained on 14 eyes not wearing contact lenses by Millodot (1972) are reproduced in Fig 1 This effect of soft lenses on corneal sensitivity is in good accord with the data of Baily & Carney (1973) who showed that the corneal oedema subsided within 2 hours after wearing soft lenses It was impractical to keep each subject for more than 9 hours for this experiment but two of them stayed for 2 hours after removing their lenses and corneal sensitivity for both subjects was found to be greater after this length of time than after 1 hour

From the present study and that of Bailey & Carney (1973) it can be inferred that corneal sensitivity appears to be inversely related to the amount of corneal oedema a fact already noted in glaucoma (Boberg Ans 1955) and in physiological variations such as the increase in corneal sensitivity throughout the day (Millodot 1972) while the cornea becomes thinner (Gerstman 1972)

Although there is a reduction of oxygen transmission through soft lenses (Hill & Augsburger 1971) it appears that this interference is not sufficient to alter normal corneal thickness (Fatt & St Helen 1971 Morrison & Edelhausser 1972) since the cornea remains unaltered until it is deprived by a critical value of the oxygen tension (Polse & Mandell 1970) Thus the corneal thickness and consequently the reduction of corneal sensitivity induced by soft lenses may be accounted for by the fact that evaporation of the tear layer is most likely hindered which reduces its concentration a fact known to give rise to corneal swelling (Cogan 1941)

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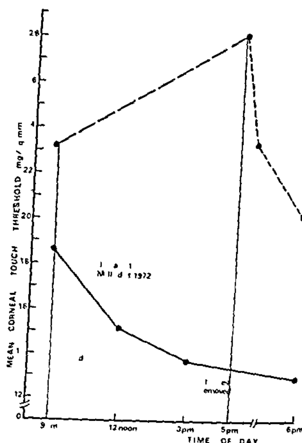


Fig 1
Threshold of corneal sensitivity to touch as a function of the time of day and the wearing of hydrophilic lenses

Discussion

One possible reason why it was possible in this experiment to demonstrate the effect of soft lenses was because the sensitivity was measured at intervals after the lens had been removed This was not the case in the experiment of Knoll & Williams (1970) and Larke & Sabell (1971) Moreover the method used in the present experiment differs from these two studies in that the aesthesiometer is rigidly mounted and mechanically handled rather than held by hand Nevertheless the reduction in corneal sensitivity induced by soft lenses is indeed a great deal less than what has been observed with hard lenses (Millodot 1971)

After 1 hour the corneal sensitivity threshold is lower than it was in the morning This is indeed explicable by the fact that corneal sensitivity increases

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A FAMILY WITH VITELLIFORM MACULAR DYSTROPHY

Best's Disease

BY

JAN KAARE BRINK

In a family of 84 members macular lesions were observed in 16 out of 75 examined individuals. The typical egg yolk lesion was seen in three cases while the remaining bore evidence of the polymorphous nature of the disease. Electro-oculography (EOG) was performed on 11 affected individuals. EOG showed pathological changes in 10 of these. In one case the changes were localized to an eye which ophthalmoscopy had shown to be normal. EOG was performed on a further nine members of the family in whom ophthalmoscopy was normal. The values were subnormal in four of these. The significance of EOG in cases of macular degeneration is accentuated. Fluorescein angiography in colour was performed on two individuals. Hepatolenticular degeneration (Wilson's disease) had previously been diagnosed in members of one branch of this family. A possible connection between Best's and Wilson's diseases is advocated in the discussion.

Key words: Best's disease – electro-oculography – fluorescein angiography – hepatolenticular degeneration – macula – retina – vitelliform dystrophy – Wilson's disease

Vitelliform macular dystrophy is a hereditary autosomal dominant disease ophthalmoscopically localized to the macula. The lesion is usually bilateral. It is described as a circular or oval, well defined lesion of 1/2–2 disc diameters either of egg yolk appearance or greyish and atrophic. The visual acuity

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A FAMILY WITH VITELLIFORM MACULAR DYSTROPHY

Best s Disease

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In a fam ly of 84 members macular lesions were observed in 16 out of 75 examined individuals The typical egg yolk lesion was seen in three cases while the rema ning bore evidence of the polymorphous nature of the disease Electro oculography (EOG) was performed on 11 affected indivi duals EOG showed pathological changes in 10 of these In one case the changes were localized to an eye which ophthalmoscopy had shown to be normal EOG was performed on a further nine members of the family in whom ophthalmoscopy was normal The values were subnormal in four of these The significance of EOG in cases of macular degeneration is accen tuated Fluorescein angiography in colour was performed on two indivi duals *Hepatolenticular degeneration (Wilson s disease)* had previously been diagnosed in members of one branch of this family A possible con nection between *Best s and Wilson s d seases is advocated in the discussion*

Key words Best s d sease – electro oculography – fluorescein angiography hepat lenticular degenerati n – macula – retina – vitelliform dystrophy Wilson s disease

Vitelliform macular dystrophy is a hereditary autosomal dominant disease ophthalmoscopically localized to the macula The lesion is usually bilateral It is described as a circular or oval well defined lesion of 1/2–2 disc dia meters either of egg yolk appearance or greyish and atrophic The visual acuity

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is generally satisfactory in the early stages while central scotomata and red green defects may develop in later stages Pathognomonic changes are demon strable by electro oculography (EOG)

Since 1905 when Best published his report on eight cases of a hereditary macular disease no particular interest was attached to the matter until late in the Fifties when reports on new aspects of Best s disease appeared in the lit erature parallel with the introduction of electroretinography EOG and fluores cein angiography

It is the purpose of the present publication to demonstrate the polymorphous nature of the disease and the value of EOG as a method by which a differential diagnosis can be established

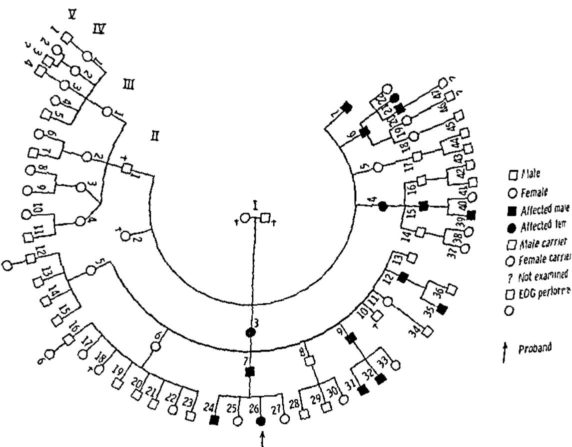


Fig 1

Pedigree of a Danish family with vitelliform macular dystrophy (Best s disease) Hepa tolenticular degeneration (Wilson s disease) occurred in family *IV 16–23* † denotes individuals who had died before the author became acquainted with the family

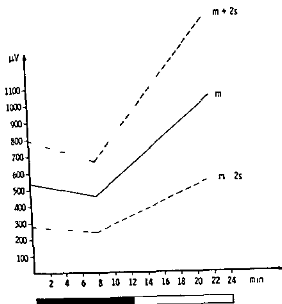


Fig 2

Electro oculcgramme Normal findings Time in minutes is plotted along the abscissa the ampl tude in µV being plotted along the ordinate. The dark column represents the period of dark adaptation the white column represents the period of light adaptation.

Material and Method

Vitelliform macular dystrophy was observed in a Danish family on whom no previous data had been published The family comprised 84 members six of whom had died at the time of investigation (Fig 1) Among the 78 members still alive were 36 females and 42 males

Ophthalmoscopy revealed lesions in 16 individuals 4 females and 12 males The sex difference is not statistically significant The typical egg yolk lesion was in evidence in only three of the affected individuals Twenty seven individuals were examined in hospital In addition to ophthalmoscopy the visual acuity was measured perimetry and EOG were performed and the colour vision was tested by Ishihara s plates Fundus photography and fluorescein angiography in colour were applied in some cases Ophthalmoscopy of the remaining members of the family was performed in their homes

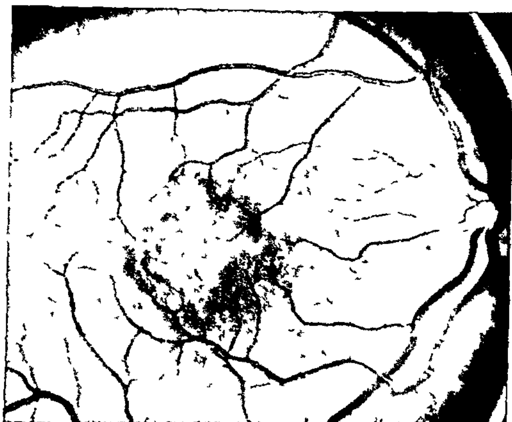


Fig 5
Vitelliform macular dystrophy "scrambled egg" stage, case IV 26, right eye of the patient

The proband was a 12 year old girl (IV 26) who on account of impaired vision was referred to an ophthalmological unit. Even though the major part of the family lives in an isolated fishing community intermarriage has not occurred. The extremely rare hepatolenticular degeneration (Wilson's disease) occurred in members of one branch of the family (IV 16–23). Symptoms of the eye disease were not observed in any of these except that EOG showed subnormal values in one individual (IV 17).

EOG

EOG was carried out in accordance with the principles outlined by Arden, Barrada & Kelsey (1962). Metal electrodes 10 mm in diameter and 2.5 mm thick were by means of electrode jelly and waterproof adhesive tape arranged in the canthi of the patient's eyes after the skin had been cleansed with benzine. Potentials were recorded by a.c. amplification on a Mingograph 34 with a time constant of 5 sec. The patients moved



Fig 4

Vitelliform macular dystrophy stage of incipient atrophy fluorescein angiography in colour case IV 6 left eye of the patient

their eyes between two fixation points thereby establishing a deflection of movement of 30° and an equivalent deflection of the potential The pupils were not dilated Each test started by recording the pre adaptation value in ordinary room lighting (60–70 lux) The procedure was followed by a dark adaptation period of 12 min and a light adaptation period of 1? min (1500 lux) Every 60 sec a curve was plotted throughout 10 sec A control series of normal subjects was required in order to estimate borderline values Sixteen subjects in the age group ?3–53 years were examined (6 females and ?? males) In the latter series the mean value of the L/D ratio (ratio of the highest value in light light peak and the lowest value in darkness dark trough in per cent) was in the range of 243 ± 32 (?s) in females and 221 ± 4? (s) in males The sex difference is not statistically significant Adams (1973) observed a statistically significant sex difference There was correlation between deflections of the potential in the right and left eyes As regards the right eyes the mean value of the L/D ratio was in the range of ?9 ± 44 (s) in females and males (Fig 2) while the equivalent mean value applying to left eyes was ??8 ± 34 (s) In the case of right eyes the dark trough was found in the range of 459 microvolt ± ?1? (?s) and the light peak in the range of 1047 ± 49 (s)

Case Histories

In the following some characteristic cases will be presented

Case IV,26

Female aged 1? ?r band. The girl was admitted to hospital complaining of poor ?n The visual acuity of her right eye was >0 67 3 50 sph 1 50 cyl (90) Ophthalmoscopy disclosed a circular reddish lesion measuring 3 4 disc diameter sur

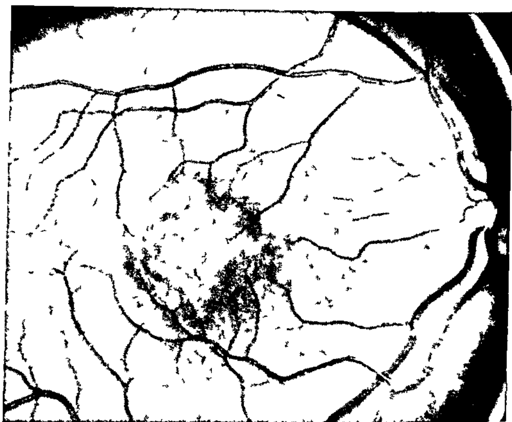


Fig 3
Vitelliform macular dystrophy scrambled egg stage case IV 26 right eye of the *patient*

The proband was a 12 year old girl (IV 26) who on account of impaired vision was referred to an ophthalmological unit Even though the major part of the family lives in an isolated fishing community intermarriage has not occurred The extremely rare hepatolenticular degeneration (Wilson s disease) occurred in members of one branch of the family (IV 16–23) Symptoms of the eye disease were not observed in any of these except that EOG showed subnormal values in one individual (IV 17)

EOG

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Fig 6

Vitelliform macular dystrophy scrambled egg stage case III 20 right eye of the patient

Case III,20

Male aged 4 The patient had no visual complaints The visual acuity of the right eye was < 1 0 1 50 sph Ophthalmoscopy showed an oval greyish yellow irregularly outlined non elevated mottled macular degeneration measuring 3/4 disc diameter (Fig 6) Red green defects were exposed by interpretation of Ishihara s plates EOG showed a L D ratio of 105

The visual acuity of the left eye was 1 0 1 50 sph Ophthalmoscopy showed normal conditions Interpretation of Ishihara s plates was correct but EOG showed a L/D ratio of 105

Case III 7

Male aged 41 The patient had noticed slightly impaired vision The visual acuity of the right eye was < 0 33 3 00 sph Ophthalmoscopy showed an almost white oval central lesion measuring 1 4 disc diameter it was sharply demarcated by a line of black pigmentation The latter was surrounded by a circular depigmented area measuring 1 disc diameter also demarcated by a pigmented zone. Juxtapapillarily a white degenerative zone was observed at the upper temporal border Interpretation of some of the Ishihara plates was found to cause certain difficulties

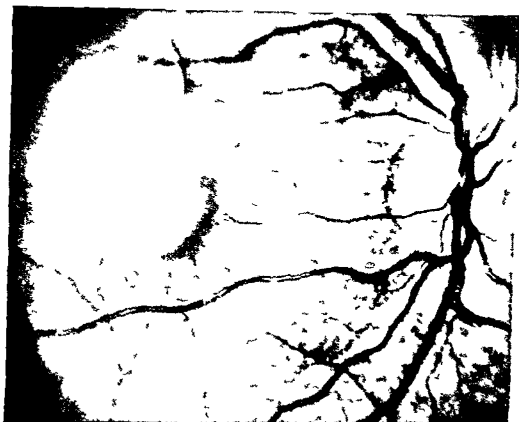


Fig 5
Vitelliform macular dystrophy vitelliform lesion case IV 32 right eye of the patient

rounded by a pigmented zone A yellowish-white area was observed centrally in the lesion (Fig 8) EOG showed a L D ratio of 110 % .

The visual acuity of her left eye was 0 67 + 5 00 sph Ophthalmoscopy showed a yellowish red slightly oval well defined lesion measuring 1 disc diameter (Fig 4) The lesion was slightly elevated It was not of a homogenous appearance but comprised paler and darker areas Fluorescein angiography in colour showed strong fluorescence of the lesion with a distinct oval configuration In an area larger than the lesion seen by ophthalmoscopy the background fluorescence was very faint LOC showed a L D ratio of less than 100 % (Fig 11) Interpretation of Ishihara s plates was correct

Case IV,32

Male, aged 7 The boy had no visual complaints The visual acuity of both eyes was 0 67 + 2 00 sph Well defined homogenous cysts of a yellow colour were observed in both eyes measuring 1 disc diameter in the right and 1½ disc diameter in the left The cysts were slightly elevated and of a characteristic dim appearance best described as sun through fog

The fundus of the right eye is illustrated in Fig 5



Fig 6
Vitelliform macular dystrophy scrambled egg stage case III 20 right eye of the patient

Case III 20

Male aged 24 The patient had no visual complaints The visual acuity of the right eye was < 1 0 1 0 sph Ophthalmoscopy showed an oval greyish yellow irregularly outlined non elevated mottled macular degeneration measuring 3 4 disc diameter (Fig 6) Red green defects were exposed by interpretation of Ishihara s plates EOG showed a L D ratio of 105

The visual acuity of the left eye was 1 0 + 1 50 sph Ophthalmoscopy showed normal condition Interpretation of Ishihara s plates was correct but EOG showed a L/D ratio of 105

Case III 7

Male aged 41 The patient had noticed slightly impaired vision. The visual acuity of the right eye was < 0 33 3 00 sph. Ophthalmoscopy showed an almost white central lesion measuring 1 4 disc diameter it was *sharply demarcated by a line* of black pigmentation The latter was surrounded by a circular depigmented area measuring 1 disc diameter also demarcated by a pigmented zone Juxtapapillarily a white *degeneration zone was observed at the upper temporal border* Interpretation of some of the Ishihara plates was found to cause certain difficulties



Fig 7
Vitelliform macular dystrophy pseudohypopyon case III 7 left eye of the patient

The visual acuity of the left eye was > 0 5 + 2 50 sph Ophthalmoscopy disclosed a circular lesion measuring 1 disc diameter divided by a horizontal line of demarcation in which the upper portion was reddish and slightly mottled in appearance (Fig 7) After the patient *had been lying on one side for a few minutes the horizontal line was seen to be obli*quely displaced thus suggesting a liquid content in the cyst

The above mentioned difficulties involved in interpretation of Ishihara s plates were encountered EOG showed a L D ratio of 100 %

Case II 3

Female aged 71 The visual acuity of the right eye was 0 1 + 2 25 sph + 0 75 cyl (130°) Ophthalmoscopy showed a diffuse poorly delimited central degeneration including yellowish white irregularly depigmented areas (Fig 8) Above this a dark reddish area was seen to cover the foveal region Small white formations were observed in the surrounding area

The visual acuity of the left eye was 0 15 + 2 25 sph + 1 00 cyl (100) Fundus examination showed a rather severe disorganization of the macular structure in which choroidal vessels were visible (Fig 9)



Fig 8
Vitelliform macular dystrophy atrophic stage case II 3 right eye of the patient

Case III 12

Male aged 54 The visual acuity of the right eye was 1 0 + 1 00 sph that of the left w s 1 0 1 0 sph The patient had no visual compla nts Both macular areas showed well defined c rcular lesions n easuring 1 s disc diameters and white spots of various size (F g 10)

EOG of both ey s sh wed L D ratios of 100 % it applies to both eyes that interpretat on f Ish ha a s plates caused certain difficulties

It applies to cases IV 1? (male aged 19) IV 14 (male aged 15) IV 15 (male aged 11) IV 40 (female aged 4) IV 41 (male aged 6) and IV 42 (male aged 4) that the foveal reflexes were surprisingly weak and might even be absent the macular area was surrounded by a larger coarsely pigmented salt and pepper zone Finally minor scattered yellowish white spots in the macular areas were observed in cases III 1 (female, aged 45) and IV 1 (female aged) EOG findings were normal in the former case (L/D ratio 2..3 % in the



Fig 9
Vitelliform macular dystrophy cicatricial changes case II 3 left eye of the patient

right eye 240 % in the left) EOG values were subnormal in the latter case (L/D ratio 173 % in the right eye 189 % in the left)

Electro oculography

EOG was performed on 11 affected individuals The potential did not change in 10 of these or changes were only faint during dark adaptation as well as during light adaptation Stimulation by light even provoked an inverse reaction in the proband (Fig 11) In case IV 35 EOG was normal even though the other wise normal structure was marked by yellowish discolouration centrally in the retina In case III 20 EOG of the left eye was pathological even though oph thalmoscopy had shown normal conditions

EOG was performed on nine apparently healthy members of the family Pathological or subnormal values were obtained in case IV 1 (both eyes 173 % and 189 %) in case IV 3 (left eye 190 %) in case IV 5 (left eye 192 %) and in case IV 17 (left eye 193 %)



Fig 10

Vitelliform macular dystrophy atrophic stage shrivelling of the cyst case III 17 right eye of the patient

Associated diseases

In conformity with findings in the family originally described by Best refraction anomalies were also observed in several patients in the present series the anomalies included hypermetropic astigmatism and hypermetropia which were seen in 5 and 17 individuals respectively Myopic astigmatism was observed in one patient (case IV 17) and convergent squint was manifest in three (cases III 11 IV 11 and IV 34)

One member of family IV 16–23 had died from Wilson s disease The correct diagnosis was not established until autopsy This diagnosis has later been established in cases IV 1 and IV 19 both patients received treatment by $\beta\beta'$ dimethylcysteine for 4 years At the time when this treatment was commenced in 1963 (Gregersen & Frederiksen 1961) a greenish brown Kayser Fleisher *ring* was observed in both patients after treatment for 1 year the ring remained unchanged In pection in the course of the *present investigation did not* disclose deposits in the cornea of these two patients.

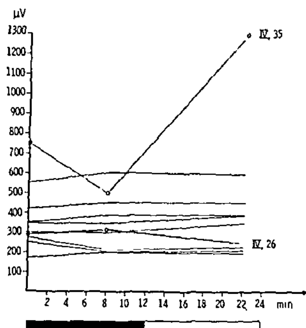


Fig 11

Electro oculography of 10 patients presenting vitelliform macular dystrophy Time in *minutes is plotted along the abscissa the amplitude in µV being plotted along the ordinate* Response was normal in only one patient (case IV 35) The proband (case IV 26 *left eye)* was the only patient in whom inverse reaction was provoked during light adaptation

Discussion

According to the findings in the present study the disease must be characterized as a hereditary autosomal dominant disease Statistical evidence of a reduced penetrance is not provided as the disease was found to be manifest in 16 out of 35 children born of affected parents (45 7 %)

Thiel & Behnke (1971) suggested a classification into stages according to the ophthalmoscopical aspect of the lesion Indeed such classification is purely arbitrary Smooth interstage transitions may be encountered Hence the following classification is suggested on the basis of findings in the family here discussed

1) Previtelliform stage
2) Vitelliform lesion
3) Incipient atrophy or a pseudohypopyon
4) Scrambled egg stage
5) Atrophic stage

1) *Previtelliform stage* This term can be applied to case III 20 EOG showed severe anomalies (L/D ratio 108 %) in the patient s left eye which by ophthalmoscopy had appeared to be normal The visual acuity was 1 0 and interpretation of Ishihara s plate was correct François et al (1967) and Mikawa & Tamura (19?) have each described one case in which EOG had disclosed changes before the lesion had become visible The postulation that lesions had been visible but later disappeared without leaving any traces seems hardly probable even though one case of this type has been reported by Deutman (1969) The cases of monocular vitelliform dystrophy described by Bussey & Berkley (1950) as well as those described by Friedenwald & Maumenee (1951) may be commensurate with case III 20 in the present series thus suggesting that it is not a *matter of a monocular lesion* Follow up examinations of the patients studied by the above authors have not been reported

Defective colour vision was not in evidence

2) *Vitelliform lesion* This term covers the homogenous circular or slightly *transverse oval yellowish well defined and elevated foci measuring up to 1 1/2* disc diameter This type of lesion was seen in three members of the family here discussed (case III 21 (right eye) case IV 32 (both eyes) and case IV,39 (both eyes)) (Fig 5) The lesion has a characteristic dim appearance excellently described as sun through fog Red green defects are not manifest in any of the cases in this group

3) *Incipient atrophy* The lesion is still circular in shape and well defined Paler and darker areas alternate it is inhomogenous in appearance and of a reddish or yellowish orange colour the dim appearance is replaced by distinct changes *This type of lesion was manifest in the left eyes of two patients* (cases IV 26 (Fig 4) and IV 31) Red green defects were not demonstrable in these cases The specific type of lesion involving the left eye of one patient (case III , Fig) is called a pseudohypopyon by some authors (Remky et al 1965 Krill et al 196? François 19 1a) The phenomenon indicates that there is still a limiting membrane

4) *Scrambled egg stage* Demarcation of the lesion is less distinct at this stage and it is fragmented in appearance though still circular or transverse oval in shape Not infrequently the central area is seen to be surrounded by marked pigmentation the intensity of which is weakened towards the periphery Figs 3 and ? illustrate this stage This type of lesion was also seen in the right eye of one patient (case IV 31) and in the left eye of another (case IV 24)

Red green defects are not yet constant findings

5) *Atrophic stage* Destructive processes and mottled pigmentation are seen

together with whitish yellow formations encircling the macular region (Fig 5) Two patients belonging to this group (case II 6 male aged 62 and case III 7 male aged 41) presented juxtapapillarily whitish though not well defined degeneration The phenomenon may be interpreted as an abortive form of multiple vitelliform cysts (Deuden 1966, 1972, Remky & Kolbl 1971) The youngest patient in whom atrophic changes were in evidence was 18 years old (case IV,24 right eye)

Rupture of the cyst or shrivelling of the latter may be responsible for the transition into the atrophic stage. In the case of rupture demarcation will probably be more irregular whereas the lesion will remain circular in shape in the case of shrivelling *The cyst may have ruptured in case II 3 (Figs 8 and 9) in case* II 4 (right eye) in case III 7 (right eye) and in case IV 24 (right eye) Shrivelling of the cyst may be in the form illustrated in Fig 10 In this particular case the visual acuity was 1 0 including correction but in most cases of a possible rupture of cysts it would be about 0 1 Haemorrhage and traction folds were in evidence in the left eye of the proband s paternal grandmother who was 71 years old (case II 3) and in the right eye of a 66 year old woman (case II 4)

All patients in this group presented red green defects

In brief the vitelliform lesions are encountered in children and in young individuals while the atrophic stages are seen mainly in older individuals the *above mentioned case (IV 24) being the only exception to the rule*

The illustrations give an impression of the polymorphous nature of the disease The diagnosis is not easily established except in cases of typical lesions. A diagnosis can hardly be established on the basis of the previous medical histories of members of the family since complaints of poor vision were only few A reliable diagnosis is most easily established by means of EOG (Grall & Bertrand 1973)

Findings by EOG at early stages while visual acuity still remains unaffected are not seen to deviate from those obtained by EOG at later stages when the lesion has atrophied and severe functional disturbances have become manifest The diagnostic value of EOG in cases of central dystrophies is accentuated by the fact that only few typical lesions were encountered in patients in the present series a feature emphasized also by Levy & Chalvignac (1970) The EOG showing normal conditions in one affected individual (Fig 11) is not readily explicable but similar phenomena have been observed by other investigators *(François 1971b and Deuden 1972)*

The findings obtained in the present study by means of fluorescein angiography are in conformity with those previously reported by Morse & MacLean (1968) and by François & De Laey (1970)

It emerges distinctly from the pedigree (Fig 1) that the ophthalmoscopically demonstrable lesions are encountered in individuals who in the pedigree are classified to the right If however the subnormal (pathological) EOG values were included they would be encountered in the apparently healthy individuals included in the pedigree to the left. This is indicative of an existence of carriers cf Deutman (1969) The greatest interest is therefore attached to one patient (case IV 17) a carrier who was suffering also from Wilson s disease If the electro oculographic criterion applying to carriers is to be maintained it seems rather surprising that EOG showed normal conditions in the parents of these carriers otherwise it must be established that EOG of carriers occasionally may show normal conditions bearing in mind that parents of carriers also must be carriers

On the basis of findings in the family discussed here it must be taken into consideration that there may be a connection between Best s and Wilson s diseases Furthermore it does not seem inconceivable that the vitelliform lesion may respond to treatment by $\beta\beta$ dimethylcysteine The EOG values were subnormal in one patient (case IV 17) who had received this drug for four years'

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EFFECTS OF A FALL IN THE INTRAOCULAR PRESSURE LEVEL ON THE PERIPAPILLARY FLUORESCEIN ANGIOGRAM IN CHRONIC OPEN ANGLE GLAUCOMA

BY

LEILA LAATIKAINEN and PEKKA MÄNTYLÄ

The effects of a long term fall in intraocular pressure on the fluorescein angiographic findings of the disc and the peripapillary area were studied in 50 eyes with chronic open angle glaucoma by comparing angiograms taken before and 1 to 6 months after a glaucoma operation After a remarkable fall (0 mmHg or more) in the intraocular pressure level obvious narrowing was seen in the main retinal vessels Filling defects of the peripapillary choroid or disc fluorescence were not clearly influenced by a decreased intraocular pressure level thus indicating the presence of permanent anatomic changes in the peripapillary choroidal vasculature Fluorescein angiography with the technique used cannot therefore be regarded as a suitable method for evaluating the safe pressure level in individual eyes

Key words open angle glaucoma – choroidal circulation – fluorescein angiography – peripapillary circulation – trabeculectomy

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The effects of a long term fall in intraocular pressure on the fluorescein angiographic findings of the disc and the peripapillary area were studied in 50 eyes with chronic open angle glaucoma by comparing angiograms taken before and 1 to 6 months after a glaucoma operation After a remarkable fall (?0 mmHg or more) in the intraocular pressure level ob *vious narrowing was seen in the main retinal vessels Filling defects of the* peripapillary choroid or disc fluorescence were not clearly influenced by a decreased intraocular pressure level thus indicating the presence of permanent anatomic changes in the peripapillary choroidal vasculature Fluorescein angiography with the technique used cannot therefore be regarded as a suitable method for evaluating the safe pressure level in *indiv dual eyes*

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thal et al 1970 Swietliczko & David 1970) A slowing up of the entire choroidal filling in glaucomatous eyes has been found in fluorescein angiographic studies (Rosen & Boyd 1970 Spaeth 1971) In addition sharply outlined areas of under filling in the peripapillary choroid have been revealed as typical findings in chronic glaucoma with visual field defects (Blumenthal et al 1971 Laatikainen 1971 Best et al 1972)

It has been considered that fluorescein angiography of the disc and the peripapillary area performed at various pressure levels would prove a valuable method for estimating the safe level of intraocular pressure in individual eyes (Spaeth & Vacharat 1972) In this work the effects of a long term fall in chronic intraocular hypertension on peripapillary vasculature were studied by comparing fluorescein angiographic findings of the disc and the peripapillary area in the same glaucomatous eyes before and after a glaucoma operation

Material and Methods

The material comprised 50 eyes with chronic open angle, simple or capsular glaucoma on which trabeculectomy was performed between December 1972 and November 1973 at the University Eye Clinic in Helsinki In each eye at least two fundus angiographies were performed one before and the other from 1 to 6 months after the operation All angiographies were carried out with the routine method using a Zeiss fundus camera and 5 cc 10% fluorescein injected into the antecubital vein (see Laatikainen 1971) The pupils were dilated with 1% cyclopentolate and 10% phenylephrine The pictures were taken at 1 to 2 sec intervals The intraocular pressure was measured with the Goldmann applanation tonometer after each examination

The angiograms of each eye obtained at different levels of intraocular pressure were compared and possible changes in the retinal vessels disc fluorescence and filling of the peripapillary choroid were observed Findings of the peripapillary choroid were correlated with the severity of glaucoma Therefore the eyes were grouped into four categories according to the amount of cupping and the visual field defect Grade 1 no cupping normal central and peripheral fields (10 eyes) Grade 2 an early glaucomatous cupping isolated paracentral or Bjerrum scotomas (27 eyes) Grade 3 an advanced glaucomatous cupping a large quadrant defect or only a central remnant left (5 eyes) and Grade 4 an advanced glaucomatous cupping at most an eccentric remnant left of the visual field (5 eyes)

Results

In 17 of the 50 eyes on which trabeculectomy was performed fundus angiography was not successful or was of poor quality due to simultaneous cataract and poor dilation of the pupil All five eyes with Gr 4 glaucomatous lesions belonged to this group

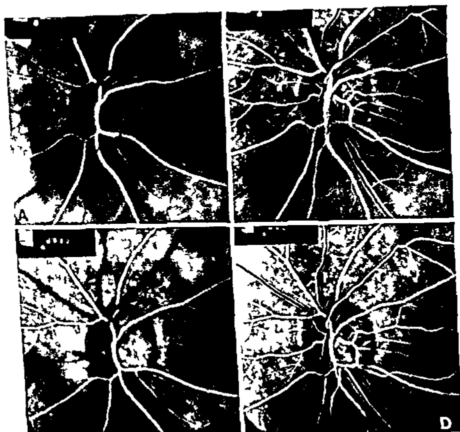


Fig 1

A 50 year old man with chronic simple glaucoma. In the left eye, glaucomatous cupping of the disc and paracentral defects in the visual field are present Fluorescein angiography taken at the intraocular pressure level of 50 shows typical filling defects of the peripapillary choroid (Figs A B) Postoperative angiography 5 months later taken at a pressure level of 1 shows marked narrowing of the retinal vessels especially arterioles and underfilling of the peripapillary choroid seems to be at least as pronounced as it was at the higher pressure level (Figs C, D)

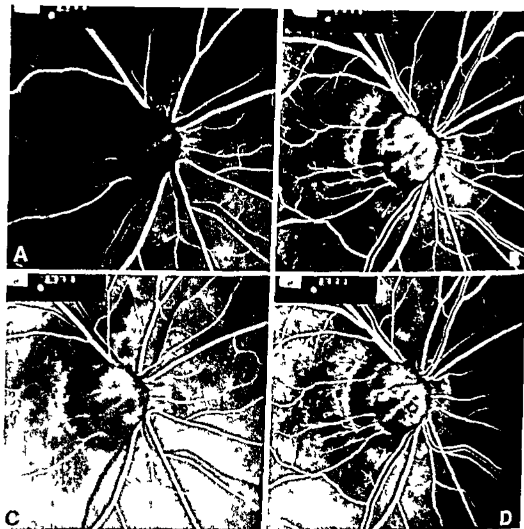


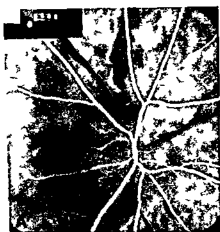
Fig 2

A 53 year old man with chronic simple glaucoma Fluorescein angiography shows underfilling of the peripapillary choroid in the right eye with deep glaucomatous cupping of the disc and large visual field defects Comparison of the preoperative (Figs A B) and postoperative (Figs C D) angiograms taken at the pressure level of 47 and 21 respectively shows that lowering of the intraocular pressure causes narrowing of the retinal vessels but no noticeable effect on the choroidal filling can be seen

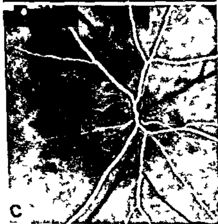
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Fig 3 (opposite page)

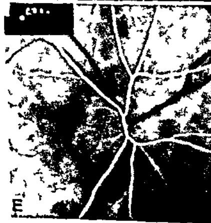
A 60 year old man with uniocular capsular glaucoma Fluorescein angiography of the glaucomatous right eye shows underfilling of the peripapillary choroid Comparison of the angiograms taken at the preoperative pressure level of 40 (Figs A B) with those taken 1 month postoperatively at the pressure level of 10 (Figs C D) and 6 months later at the pressure level of 15 (Figs E F) indicates that lowering of the intraocular pressure causes permanent narrowing of the retinal vessels especially arterioles It also seems as if underfilling of the peripapillary choroid had become more pronounced after the operation






C


D


E


F

Table I

The angiographic findings of the peripapillary choroid in the 33 eyes in which good angiograms were obtained correlated with the severity of glaucoma (Grades 1–4 of the glaucomatous lesions used are defined in the text)

Severity of glaucoma	No of eyes (per cent)			
	Gr 1	Gr 2	Gr 3	Gr 4
Normal peripapillary filling	2 (22 %)	2 (11 %)	0 (–)	0 (-)
Delayed filling of the choroid	2 (22 %)	2 (11 %)	1 (20 %)	0 (-)
Areas of underfilling in the peripapillary choroid	5 (56 %)	15 (78 %)	4 (80 %)	0 (-)
Total	9 (100 %)	19 (100 %)	5 (100 %)	0 (-)

In eight eyes with considerable fall (20 mmHg or more) in intraocular pressure after the operation marked narrowing of the main retinal vessels was seen postoperatively (Figs 1 2 and 3) In the other eyes no obvious changes could be seen in the retinal vessels except in one eye where postoperative choroidal effusion developed accompanied by congestion of the retinal vessels as well Neither could any differences be seen in the disc fluorescence In many eyes however individual disc vessels could not even be clearly seen because the pictures were focused at the level of the peripapillary retina

Fluorescein angiographic findings of the peripapillary choroid were graded as follows 1 even filling of the choroid beginning at the same time or before filling of the retinal vessels (4 eyes) 2 delayed filling of the peripapillary choroid (5 eyes) and 3 sharply outlined areas of underfilling in the peripapillary choroid as far as the disc margin (24 eyes) The angiographic findings of the peripapillary choroid were correlated with the severity of glaucoma (Table I)

In most cases peripapillary filling defects were seen at the upper or lower temporal side of the disc roughly corresponding with the vascularity and cupping of the disc It was however possible to find normal peripapillary fluorescence adjacent to an avascular part of the disc as is seen at the nasal side of the disc in Fig 1 indicating that these adjacent areas were supplied by different branches of the posterior ciliary arteries

A postoperative fall in intraocular pressure did not result in disappearance of the choroidal filling defects in any of the eyes examined (Figs 1 2 and 3) In some cases underfilling of the peripapillary choroid seemed even more pronounced after surgery (Figs 1 and 3)

Discussion

Narrowing of the retinal vessels after lowering of the intraocular pressure as well as dilation of them during induced intraocular hypertension has earlier been revealed and explained as evidence for autoregulation in the retinal circulation (Dobree 1956 Ross Russell 1973) The present study suggests that this regulatory mechanism seems to work not only in acute but in chronic conditions as well The efficient autoregulation of the retinal vessels in monkey eyes has also been confirmed with the labelled microsphere method by Alm & Bill (1973) whereas autoregulation of the blood flow in the choroid seemed to be lacking

Results of the present investigation support the earlier observations indicating that sharply outlined sectoral and circumpapillary filling defects in the peripapillary choroid as far as the disc margin are related to the level of the intraocular pressure and the severity of glaucoma (Blumenthal et al 1971 Laatikainen 1971) This points to the part played by the ciliary circulation in the pathogenesis of the optic disc cupping and of the visual field defects In the present material peripapillary filling defects were however also seen in five of the nine eyes in which no cupping of the disc or visual field defect could be found This shows that ischaemia of the peripapillary retina due to an insufficient blood flow in the choroidal branches of the posterior ciliary arteries does not yet result in glaucomatous field defects but that there must presumably be degeneration of the nerve fibers due to extension of the circulatory deficiency to the circle of Zinn and Haller and to the optic disc branches

It has been reported that peripapillary filling defects which were seen in most glaucomatous eyes during acute elevation of intraocular pressure filled however with fluorescein when intraocular pressure was reduced below 50 mmHg (Best et al 1972) In all the cases presented here filling defects of the peripapillary choroid had not disappeared even after remarkable and long term fall in intraocular pressure This indicates that initial functional disturbances in the peripapillary choroidal circulation had already become permanent defects in the choroidal vasculature

The method of angiography used is not precise enough for evaluation of the incipient functional changes in the disc or peripapillary circulation caused by moderate ocular hypertension and is therefore not a suitable method for the determination of the safe level of intraocular pressure in any individual eye. The value of fundus angiography as a routine method for revealing circulatory disturbances in glaucomatous eyes is also – as in the present material – often limited by the occurrence of a simultaneous cataract, poor dilation of the pupil or difficulties in fixation. In eyes with ocular hypertension but no glaucomatous field defects the discovery of peripapillary filling defects may, however, indicate a greater risk of developing visual field loss than if normal choroidal filling was found.

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SCHLEMM'S CANAL AND SCLERAL SPUR IN NORMAL AND GLAUCOMATOUS EYES

BY

A P NESTEROV N H HASANOVA and Y E BATMANOV

In 71 cases of primary open angle glaucoma and in 29 cases of angle closure glaucoma a sinus trabeculoectomy was performed and the biopsy specimens were studied by light microscopy A control group included 100 cadaver eyes

The anterior position of Schlemm s canal prevailed in cases of open angle glaucoma (82 %) and the posterior position of the canal was more common in cases of angle closure glaucoma (57 %)

In glaucomatous eyes Schlemm s canal was narrowed and in some cases obliterated In eyes with open angle glaucoma the mean diameter of scleral spur was 21 6 ± 4 22 microns as compared to 55 2 ± 2 76 microns in normal eyes and 70 5 ± 9 40 microns in eyes with angle closure glaucoma

In 100 eyes with open angle glaucoma and in 112 normal eyes the position of Schlemm s canal was estimated by gonioscopy The anterior position of Schlemm s canal was found in 67 % of all the cases with open angle glaucoma and in only 25 % of normal eyes

Key words primary glaucoma – histology – gonioscopy – Schlemm s canal – scleral spur

The leading role of functional blockage of the anterior chamber angle by the iris root in pathogenesis of primary angle closure glaucoma is well known Recently a concept of functional blockage of Schlemm s canal by its inner wall in most cases of open angle glaucoma was advanced (Nesterov 1968 1970

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Suson & Schultz 1969) This concept draws closer primary open angle and angle closure glaucomas They are considered to be diseases which start from the functional blockage of the aqueous outflow pathways

The fact that the canal of Schlemm may be compressed under certain conditions was demonstrated both by experiments (Dannheim & Barany 1968 Ellingsen & Grant 1972 Nesterov & Batmanov 1972) and by clinical observations (Nesterov 1968 Suson & Schultz 1969)

The mechanism of the blockage of Schlemm's canal is not yet clear The outside displacement of the trabecular wall is caused by the increase of the pressure gradient between the anterior chamber and the canal of Schlemm. The reason for this is supposed to be the increase of the outflow resistance in the trabecular meshwork However only the usual ageing changes of the latter were found in the eyes with early open angle glaucoma (Ashton 1960 Fink Felix & Fletcher 1972) Rohen & Witmer (1972) described plaques of homogeneous osmiophilic material in the inner wall of Schlemm's canal in early open angle glaucoma but their role in the pathogenesis of the glaucomatous process is unknown

Tripathi (1972) studied four eyes with open angle glaucoma and found a decrease in size and number of the giant vacuoles in the endothelial cells lining the inner wall of Schlemm's canal However Fink et al (1972) did not discover any changes in the endothelium of the canal of Schlemm in their four cases of open angle glaucoma

In a previous report (Nesterov & Batmanov 1972) we suggested that the usual ageing changes of the trabecular wall may result in the collapse of the canal of Schlemm in eyes with anatomical predisposition The latter includes the anterior position of the canal the short scleral spur and the posterior attachment of the ciliary muscle with regard to the canal of Schlemm These features diminish the efficiency of the mechanism ciliary muscle scleral spur trabecular meshwork which maintains the lumen of Schlemm's canal in the open state

The above mentioned features were studied in glaucomatous eyes with the help of light microscopy and gonioscopy The results of this study are the subject of the present paper

Material and Methods

Specimens taken from 100 glaucomatous eyes of 93 patients were studied Primary open angle glaucoma was diagnosed in 71 cases and chronic angle

closure glaucoma in 29 cases. In each case a piece of the internal corneascleral lamina 5 mm long and 3 mm wide was excised during trabeculoectomy (Nesterov, Fedorova & Batmanov 1972) and fixed in a 10 % buffered formalin solution. Celloidin embedded blocks were prepared. Sagittal sections were cut and stained by the Van Gieson method.

A hundred presumably normal cadaver eyes taken 12 to 24 hours after death served as a control group. Celloidin embedded blocks of the anterior segment of the eyeball were prepared and the sagittal sections were stained by the Van Gieson technique. The detailed data about this group of eyes was published elsewhere (Nesterov & Batmanov 1972).

In the sagittal sections on the 12 hour meredian three parameters were measured: (1) the position of Schlemm's canal in relation to the apex of the anterior chamber, (2) the diameter of the scleral spur, and (3) the width of the lumen of the canal of Schlemm. The techniques of these measurements were described in our previous publication (Nesterov & Batmanov 1972).

In order to evaluate the influence of the operation of trabeculoectomy on the findings, this operation was performed on five cadaver eyes. Specimens as well as the remaining part of the anterior segment of the eyeballs were studied as described above. In all cases the size of the parameters in question (position of Schlemm's canal, width of its lumen and diameter of scleral spur) coincided in the sections obtained from the specimens and from the adjacent area of the anterior segment of the same eyes.

Table I

The position of Schlemm's canal in normal and glaucomatous eyes (microscopic data)

Diagnosis	The position of Schlemm's canal					
	Anterior		Middle		Posterior	
	No of eyes	%	No of eyes	%	No of eyes	%
Normal eyes	32	32	51	51	17	17
Open angle glaucoma	42	82	9	18		
Closed angle glaucoma	7	33	2	10	12	57

It was possible to evaluate the position of Schlemm's canal in 51 eyes of 71 with primary open angle glaucoma and in 21 eyes of 29 with primary closed angle glaucoma.

The position of Schlemm s canal was classified into three groups – anterior middle and posterior position In the first position the apex of the chamber angle was situated behind Schlemm s canal In the middle position the posterior end of the canal and the apex are at about the same level Part of the canal of Schlemm was behind the apex in the posterior position

Position of Schlemm s canal was also evaluated gonioscopically in 112 normal eyes of 56 adult persons and in 100 eyes of 86 patients with primary open angle glaucoma It is possible to recognise the canal of Schlemm through translucent trabecular meshwork in some eyes and in others a pigmented band is seen in the posterior part of the trabeculae

The width of the white scleral ring between the canal and the ciliary body was graded from 0 to 3 The position of Schlemm s canal was evaluated as follows grade 3 of the scleral ring corresponded to the anterior position of the canal grade 2 indicated the middle position and grades 1 or 0 corresponded to the posterior position of Schlemm s canal

Results

Microscopic data Our data on the position of Schlemm s canal in the upper segment of the eyeball are summarized in Table 1 In the control group of eyes the anterior position occurred in 32 % of all cases the middle position in 51 % and the posterior position in only 17 % The majority of eyes (82 %) with chronic open angle glaucoma have anterior position of the canal of Schlemm (Figs 1 2 and 3) The middle position was found in only 12 eyes (18 %) There was not a single case where the canal of Schlemm was in the posterior position The difference in the distribution of the position of Schlemm s canal between the control and the glaucomatous eyes is significant ($P < 0.003$)

The posterior position of the canal of Schlemm prevailed (57 %) in the eyes with angle closure glaucoma (Figs 4 and 5) The anterior and the middle positions occurred in 33 % and 10 % respectively The difference between the control group of eyes and the cases with angle closure glaucoma as well as between the latter group and the eyes with open angle glaucoma is significant ($P < 0.05$) In most specimens taken from glaucomatous eyes Schlemm s canal was narrowed or partially closed (Fig 6) In six far advanced cases it was completely obliterated Our data concerning the width of the canal of Schlemm are presented in Table II



Fig 1
Histologic section showing typical anterior position of Schlemm's canal (S c) and the posterior attachment of the ciliary muscle (c m) to the sclera in an eye with advanced open angle glaucoma Van Gieson stains (× 65)



Fig 2
The fibers of the ciliary muscle (c m) do not reach the trabecular meshwork and the canal of Schlemm (S c) which is in the anterior position A case of open angle glaucoma Van Gieson stains (× 105)



Fig 3

Anter or position of Schlemm s canal (S c) in an eye with advanced open angle glaucoma Van Gieson stains (× 65)



Fig 4

Hist l gic section showing typical posterior position of Schlemm s canal (S c) and anterior insertion of the ciliary muscle (c m) in a case with primary angle closure glaucoma Van Gieson stains (× 65)



Fig 5

Posterior position of Schlemm's canal (S c) in a patient with angle closure glaucoma *Note the* anterior attachment of the ciliary muscle (c m) to the scleral spur and to the trabecular meshwork Van Gieson stains (× 105)

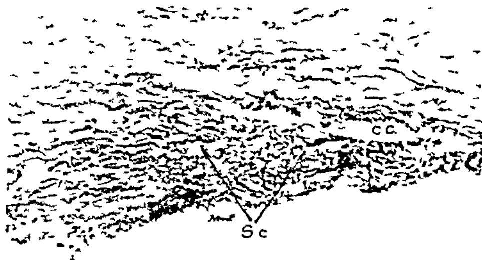


Fig 6

Drainage area in an eye with primary open angle glaucoma Schlemm's canal (S c) is narrowed and partially collapsed A collector channel (c c) appears to be widened Van Gieson stains (× 105)

Table II

The width of Schlemm s canal and the diameter of scleral spur in normal and glaucomatous eyes

Diagnosis	Width of the lumen of Schlemm s canal			Diameter of scleral spur		
	No of eyes	Mean	Mean error	No of eyes	Mean	Mean error
Normal eyes	100	24 9	1 45	100	85 2	2 6
Open angle glaucoma	68	2 7	0 42	50	91 6	4 92
Closed angle glaucoma	26	3 1	1 10	16	0 5	9 40

The means and the mean errors are given in microns



Fig 1

\ a c of early open angle glaucoma Note that the scleral spur (S s) is situated b hind the canal of Schlemm (S c) and the trabecular meshwork (Tr) Van Gieson stains (× 105)



Fig 5
Posterior position of Schlemm's canal (S c.) in a patient with angle closure glaucoma Note the anterior attachment of the ciliary muscle (c m) to the scleral spur and to the trabecular meshwork Van Gieson stains (× 105)

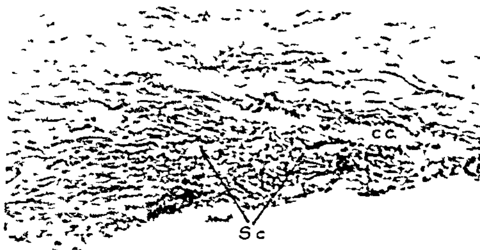


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The means and the mean errors are given in microns



Fig [illegible]

[illegible] of early open angle glaucoma Note that the scleral spur (S s) *is situated* behind the canal of Schlemm (S c) and the trabecular meshwork (Tr) Van Gieson stains (× 105)



Fig 8
Histologic section showing absence of scleral spur in an eye with early open angle glaucoma The lumen of Schlemm's canal (S c) in its posterior portion is patent and there are no marked degenerative changes in the trabecular meshwork (Tr) Van Gieson stains (× 345)

The diameter of the scleral spur was 85 2 ± 2 76 microns (mean ± mean error) in normal eyes 70 5 ± 9 40 microns in cases with angle closure glaucoma and only 21 0 ± 4 22 microns in eyes with open angle glaucoma The mean diameter of scleral spur was as much as four times less than in normal eyes ($P < 0\ 001$) In some cases with open angle glaucoma there was no scleral spur at the level of Schlemm's canal (Figs 7 and 8) In many cases with primary open angle glaucoma the tip of the ciliary muscle did not reach the



Fig 9

A case of early open angle glaucoma Note that the ciliary muscle (c m) is attached to the sclera far behind the posterior end of the canal of Schlemm (S c) Van Gieson stains (X 105)

posterior end of Schlemm s canal attaching to the sclera far behind the latter (Figs 1 2 3 and 9) In the specimens taken from the patients with angle closure glaucoma the diameter of the scleral spur was about the same as in normal eyes (Table II)

Table III

The position of Schlemm s canal in normal and glaucomatous eyes (gonioscopic data)

Diagnosis	The position of Schlemm s canal					
	Anterior		Middle		Posterior	
	No of eyes	%	No of eyes	%	No of eyes	%
Normal eyes	28	25	54	48	30	27
Open angle glaucoma	6	6	[illegible]	[illegible]	6	6

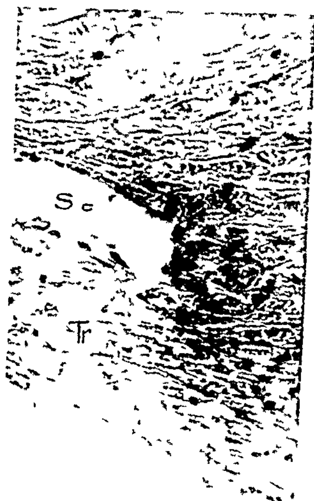


Fig 8

Histologic section showing absence of scleral spur in an eye with early open angle glaucoma The lumen of Schlemm s canal (S c) in its posterior portion is patent and there are no marked degenerative changes in the trabecular meshwork (Tr) Van Gieson stains (× 345)

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The efficiency of the mechanism ciliary muscle scleral spur trabecular meshwork depends to a certain extent on the relationship between the ciliary muscle and the trabecular meshwork In cases with anterior position of Schlemm s canal the meridional portion of the muscle may not reach the trabecular meshwork getting attached to the sclera behind the canal

The main part of the meridional fibers of the ciliary muscle is inserted into the scleral spur The wider the latter the greater the effect of the muscle on the trabecular meshwork and Schlemm s canal Besides this a rigid and well developed scleral spur protects the posterior portion of Schlemm s canal from collapse Actually in both the normal and the glaucomatous eyes the posterior portion of the canal was as a rule wider than its anterior portion

Po terior position of Schlemm s canal is more common in cases with angle closure glaucoma This seems to predispose the trabecular meshwork to be closed by the iris root, especially in eyes with a sharp apex of the anterior chamber angle (Nesterov & Batmanov 1972) Narrowing and partial collapse of Schlemm s canal in eyes with angle closure glaucoma may be explained by the subsequent sharp rise of the pressure gradient between the anterior chamber and the canal of Schlemm

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Gonioscopic data The results of gonioscopy as regards the position of Schlemm s canal are given in Table III The frequency of the anterior position of the canal was much higher (67 %) in eyes with open angle glaucoma than in normal eyes (25 %) This difference is significant ($P < 0.01$)

Discussion

No histological technique is free from artifacts However it is unlikely that the latter could change our results significantly Making use of celloidin embedded blocks and relatively thick sections we tried to diminish the degree of tissue deformation (Eliseev 1967)

It should be mentioned that estimation of the position of Schlemm s canal with the help of gonioscopy is not quite reliable as such estimation is rather subjective In some eyes extensive pigmentation of the scleral spur made it especially difficult In spite of possible mistakes gonioscopy is believed to be useful for approximate evaluation of the position of Schlemm s canal A relatively good agreement between the results obtained both by the gonioscopic and microscopic techniques (see Tables I and III) is evidence in favor of this conclusion

Relying on data presented above one may suggest that the anterior position of Schlemm s canal as well as the short and weak scleral spur are characteristic of primary open angle glaucoma The poor development of the scleral spur in eyes with chronic open angle glaucoma was observed earlier by Rohen (1969) These peculiarities seem to predispose the collapse of Schlemm s canal under certain conditions

The trabecular wall of Schlemm s canal may be regarded as a diaphragm separating the lumen of the canal from the anterior chamber The pressure on the inner surface of this diaphragm is larger than that on the outer surface This is a cause of outside displacement of the trabecular wall and the narrowing of the lumen of Schlemm s canal

The degree of the displacement is caused not only by the value of the pressure gradient but also the rigidity of the diaphragm The rigidity of the trabecular wall depends on anatomical factors

The meridional fibers of the ciliary muscle are attached to the scleral spur and to the trabecular beams Due to the constant tonus of the muscle the trabecular wall is stretched The stretching of the trabecular wall increases its rigidity and widens the lumen of Schlemm s canal

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VESICULAR TRANSPORT OF HORSERADISH PEROXIDASE ACROSS THE RABBIT CORNEAL ENDOTHELIUM

Short Communication

BY

ASBJØRN M TØNJUM

Key words vesicular transport – cornea – endothelium – peroxidase horseradish

Adult healthy albino rabbits with normal eyes were killed with an overdose of sodium pentobarbital The corneae were quickly excised and clamped between two lucite chambers which contained a nutrient solution pH 7 4 Ca^{++} Mg^{++} and glucose To the endothelial side chamber was added horseradish peroxidase Sigma type II (mol wt about 40 000 diameter about 3 nm) to concentrations of 1–9 mg ml and left for 2 5 30 60 or 120 min The corneae were processed for light and electron microscopy as described in another paper (Tønjum 1974)

Light microscopy revealed the presence of large amounts of peroxidase reaction products in the Descemet s membrane the stroma and in the epithelium up to the outermost cell layer the amount varied with the exposure time The cytoplasma of the endothelial cells was pale except for a number of dark dots and there was only a faint staining of the intercellular spaces Electron micrographs showed endocytotic vesicles within the endothelial cells containing peroxidase reaction products Some large vesicles appeared to be lysosomes

Vesicles of the corneal endothelial cells containing thorium dioxide were demonstrated by Donn Kaye Mallett & Pappas 1961 and recently vesicles with

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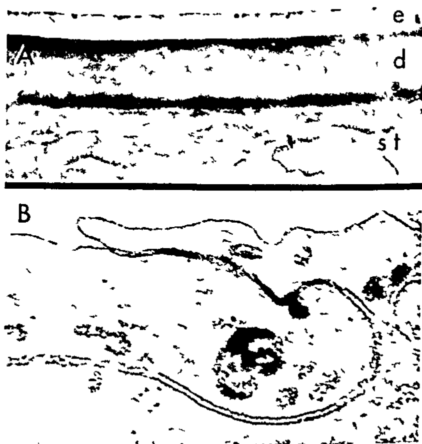


Fig 1

A Light micrograph of corneal endothelium (e) Descemet s membrane (d) and posterior part of the stroma (st) Exposure time 120 min Intensive staining of the Descemet s membrane by peroxidase reaction products A number of dark dots in the endothelial cells particularly lined up along the posterior surface × 750

B Electron micrograph of section from the same specimen showing posterior part of two endothelial cells with junctional complexes and a number of endocytotic vesicles containing peroxidase reaction products The large vesicle in the centre might be a lysosome Contrasted with alcaline lead × 60 000

horseradish peroxidase were studied by Kaye Sibley & Hoefle (1973) The exposure time of the latter study was short and attention was focussed on the intercellular spaces

The present study indicates transport of considerable amounts of the peroxidase across the apparently intact endothelium and that a major part of this transport occurred by means of endocytotic vesicles Between 30 and 60 min were required for the enzyme to move across the entire thickness of the cornea

Further studies are in progress

Little attention has been paid to the permeability of the corneal epithelium to large molecules There are conflicting views as to whether or *not serum* proteins are present in the corneal epithelium François & Rabaey 1963 Berger 1969 and others have demonstrated albumin in epithelial extracts while *Holt* & Kinoshita 1973 claimed that the corneal epithelium was devoid of serum proteins Hall Smolin & Wilson 1974 state that the traces of serum proteins which they detected could well have come from the stroma during the scraping process

Horseradish peroxidase (PO) *having* a molecular weight of about 40 000 and an approximate diameter of 3 nm has been widely used as a cytochemical and histochemical tracer (Graham & Karnovsky 1966) *The movement of the enzyme* may be arrested by prefixation in glutaraldehyde and visualized by acting upon hydrogen peroxidase in the presence of diaminobenzidine (*DAB*) The peroxidase reaction products (PORP) appear dark brown by light microscopy of sections stained with toluidine blue or unstained and they are electron opaque

Material and Methods

Albino rabbits weighing 3–4 kg were used They were adult and healthy and prior to experimentation their eyes were examined with slit lamp and ophthalmoscope and found to be normal

The experiments described in the present study were done *in vitro* The corneae were excised from the proptosed eyes after having killed the animal with an overdose of sodium pentobarbital and they were quickly clamped between two lucite chambers both having a volume of about 2 5 ml The surfaces of the chambers facing each other were curved to fit the rabbit corneae The chambers had circular openings on both the endothelial and the epithelial side of 6 mm in diameter without any support and they had apertures to the air as well Stirring of the solutions in both chambers was provided by means of small teflon coated magnets which were rotated at a speed of *375 rpm* Care was taken to avoid damaging and drying of the tissues No attempts were made to adjust the hydrostatic or *intraocular pressure*

The following nutrient solution was used NaCl 102 mM KCl 3 7 mM $MgSO_4$ 1 1[illegible] mM $CaCl_2$ 2 5 mM NaH_2PO_4 0 58 mM glucose 1 mg/ml adjusted to pH 7 4 with NaOH or HCl (Barany 19[illegible])

Horseradish peroxidase *Sigma type II* was dissolved in the nutrient solution to concentrations varying from 1–9 mg/ml

The University Eye Department (Head: Th. L. Thomassen, M.D.) and the Institute of Pathology, Electron Microscopic Laboratory (Head: T. Hovig, M.D.), Rikshospitalet, Oslo

PERMEABILITY OF HORSERADISH PEROXIDASE IN THE RABBIT CORNEAL EPITHELIUM

BY

ASBJORN M. TONJUM

Horseradish peroxidase has been used in the cornea as a histochemical tracer for both light and electron microscopy. When the rabbit corneae, as little damaged as possible, were mounted between two lucite chambers containing a nutrient solution and exposed to this enzyme on the epithelial side, no peroxidase reaction products were detected below the epithelial surface. When the endothelial side had been exposed for one hour or more, the enzyme had moved across the endothelium, the Descemet's membrane, the stroma and up between the epithelial cells to the *zonulae occludentes* between the cells of the most superficial layer adjacent to the tear film.

Key words: permeability – cornea – epithelium – rabbit – horseradish peroxidase.

The corneal epithelium acts as a barrier against penetration of many solutes into the eye. As to water, clinical evidence from bullous keratopathy as well as experimental data obtained by Green (1969) by posteriorly applied hydrostatic pressure indicate that the deeper layers of the epithelium are more permeable than the superficial ones. This seems to be in accordance with electropotential studies of the corneal epithelium (Ehlers 1973, Klyce 1972).

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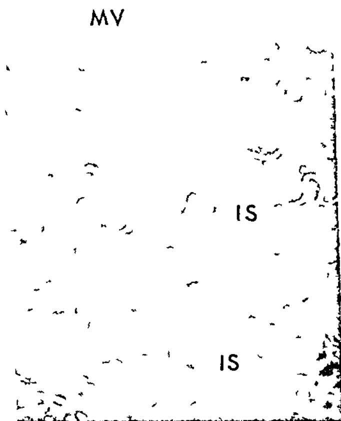


Fig 1
Superficial layers of rabbit corneal epithelium anteriorly exposed to horseradish peroxidase No peroxidase reaction products present MV microvilli IS intercellular spaces Contrasted with alcaline lead × 54 000

filled with PORP and some of the intracellular contents had disappeared These cells seemed to be dead and the presence of PORP inside the cell was most likely due to a desintegration of the cell membrane. No PORP was detected underneath these cells thus indicating that the barrier was intact (Fig 4)

PO applied on the endothelial surface

When the endothelium had been exposed to PO for 60 or 120 min a distinct and intensive staining with PORP of the intercellular spaces of the epithelium

The PO was applied to the corneal epithelium in three ways (1) PO dissolved in the nutrient solution was placed in the epithelial side chamber for 2 5 or 10 min before the corneae were removed prefixation of the tissues and processing of the PORP was started (2) The PO solution was placed in the endothelial side chamber Now the PO had to move across the entire thickness of the cornea to reach the epithelium and was therefore left in contact for 30 60 or 120 min At the end of the e periods the nutrient solutions on the epithelial side were tested for PO activity by adding DAB and H_2O_2 (3) About 0 01 ml of the PO solution 2 5 mg/ml was injected into the stroma by means of a microsyringe and a 27 gauge cannula Thus a small bleb of about 3 mm in diameter and slightly elevated was produced These corneae were excised along with some scleral tissue and were left for 60 or 120 min in the nutrient solution All experiments so far were done at 21° C

For prefixation the corneal tissues were immersed *en bloc* in cooled 2 5 % *glutaraldehyde in 0 1 M phosphate buffer of pH 7 4 for 2 hours* The tissues were left overnight in 0 1 M phosphate buffer with 5 % sucrose at 4° C The *blocks were then cut into slices of 0 5–1 mm in thickness* containing all layers of the corneae Incubation of the tissue slices was done in Tris HCl buffered DAB H O pH 7 6 for 30 min (Karnovsky 1967) The tissue slices were then washed three times in cooled distilled water postfixed in 1 % osmium tetroxide in Millonig s phosphate buffer pH 7 4 for 1½ hours dehydrated in ethyl alcohol treated with propylenoxide and embedded in Epon 812

Semi thin and ultra thin sections were cut by means of an LKB Ultratome For light microscopy sections either stained with toluidine blue or unstained were examined The ultra thin sections were either left uncontrasted or were contrasted with alcaline lead and/or uranyl acetate Electron micrographs were taken with a Siemens Elmiscope 1 A

Observations

Both the endothelial and the epithelial cells were well preserved in all preparations without any sign of lysis of the cell membranes or of the intracellular organelles except for a couple of superficial epithelial cells which appeared to be dead

PO applied on the anterior surface

When PO was applied to the anterior surface no PORP was detected under the surface of the epithelial cell layer However a couple of superficial cells were

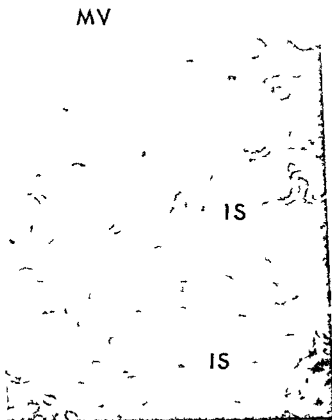


Fig 1
Superficial layers of rabbit corneal epithelium anteriorly exposed to horseradish peroxidase. No peroxidase reaction products present. MV microvilli, IS intercellular spaces. Contrasted with alcaline lead. × 34 000

filled with PORP and some of the intracellular contents had disappeared. These cells seemed to be dead and the presence of PORP inside the cell was most likely due to a desintegration of the cell membrane. No PORP was detected underneath these cells, thus indicating that the barrier was intact (Fig 4).

PO applied on the endothelial surface

When the endothelium had been exposed to PO for 60 or 120 min a distinct and intensive staining with PORP of the intercellular spaces of the epithelium



Fig 2
Light micrograph of unstained section The endothelial side of the cornea had been exposed to horseradish peroxidase for 120 min Peroxidase reaction products in the intercellular spaces up to the outermost cell layer SE superficial epithelial cells WE win cells BE basal cell layer St stroma x 750

was found by light microscopy The outlining of the cellular borders was present even between the outermost epithelial cells except for a short interval adjacent to the tear film (Fig 2) The staining was regularly visible only in the superficial half of the epithelial layer But on examining sections of the edges of the slices PORP was present at all levels of the cornea This indicates that PO was present at all levels but only visualized in the superficial part probably due to the poor penetration of DAB

Electron microscopy revealed the presence of electron opaque PORP in the intercellular spaces of the corneal epithelium except for a short interval between the outermost cells close to the epithelial surface interpreted to be a *zonula occludens* (Fig 3)

When the endothelium had been exposed to the PO solution for only 30 min no PORP was detected in the epithelium

No PO activity was detected in samples from the epithelial side chamber after 60 or 120 min

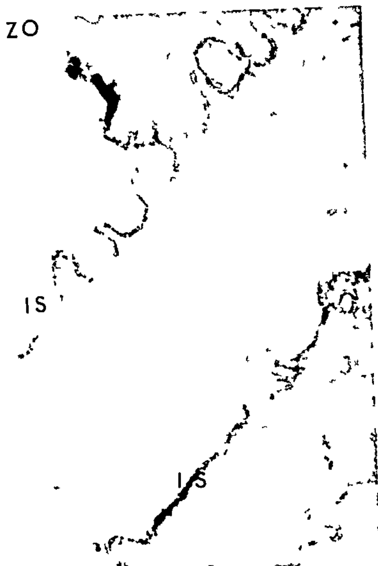


Fig 3
Superfic al cell layers of a rabbit cornea which had been exposed to horseradish per xid s n the nd thelial side for 1 0 m n *in vitro* Electron opaque material in the nter ll lar spac s (IS) except for a short interval adjacent to the surface interpreted to be a *oni la occludens* (ZO) Uncontrasted 30 000



Fig 2
Light micrograph of unstained section The endothelial side of the cornea had been exposed to horseradish peroxidase for 120 min Peroxidase reaction products in the intercellular spaces up to the outermost cell layer SE superficial epithelial cells WE wing cells BE basal cell layer St stroma × 630

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intercellular spaces at all levels of the epithelium but was prevented from entering the epithelial side chamber The only definite point of barrier was found to be at the junctional complex between the outermost epithelial cells adjacent to the tear film The superficial cells are probably completely surrounded by these tight junctions interpreted as being *onulae occludentes* Apparently this is the only location for these types of junctional complexes in the corneal epithelium On the other hand numerous desmosomes were present and they were increasing in number towards the surface in accordance with Pei & Rhodin 1971

No conclusion can be reached from this study as to whether the movement of PO in the intercellular spaces takes place by active transport or by passive diffusion

When PO was applied to the endothelial surface the enzyme required between 30 and 60 min under the present conditions to move across the entire thickness of the cornea

Large molecules at least up to the size of horseradish peroxidase may enter the epithelial layer from the stromal side If serum proteins are present in the normal corneal epithelium they have probably reached there from behind and not from the anterior side that is the tears unless some specific transport mechanisms are involved

On the other hand a lesion of the outermost epithelial cell layer would be sufficient for PO or other molecules up to that size to gain access to the corneal tissue This has relevance to the increase of permeability produced by the cationic surfactant benzalconium chloride which is commonly added to eye drops as a preservative (Tonjum 1974b)

During the normal turnover of the epithelium the cells die and are removed Most likely the superficial cells which were filled with PORP were dead before or early on during the experiments and were in the process of being removed Still there was no sign of leakage of PO to the tissue underneath This might indicate that the barrier function is established before removal of a dead cell takes place

That PO reaches the intercellular spaces of the epithelium when applied posteriorly proves that considerable amounts of PO permeate across the endothelium and the stroma under the present conditions This will be dealt with separately (Tonjum 19 4a)

Acknowledgement

Fin n ial support from the Norwegian Research Council for Science and Humanities nd from Aase and Knut T njum, Tonsberg is gratefully acknowledged.

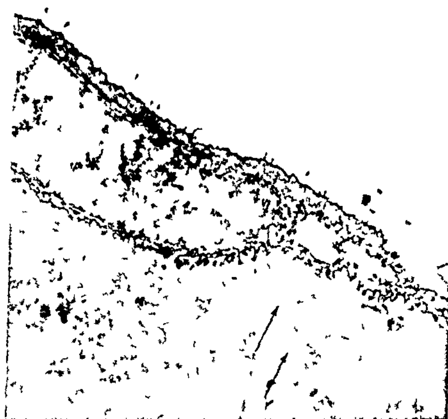


Fig 1

Dead superficial cell of rabbit corneal epithelium showing lysis of the nucleus and intracellular organelles Horseradish peroxidase had been exposed to the epithelial side Intracellular staining with peroxidase reaction products but not underneath this cell Arrows indicate intercellular spaces Contrasted with alcaline lead × 12 000

PO injected into the stroma

When PO had been injected into the stroma light and electron microscopy showed grossly the same distribution pattern of PORP in the epithelium as when it was applied to the endothelial surface However over the bleb there was some intercellular oedema with stretching of the cell membranes at the desmosomes

Comments

The present study indicates that there is a tight barrier against permeation of horseradish peroxidase across the rabbit corneal epithelium under these experimental conditions When PO was applied anteriorly it did not penetrate into the epithelium However when applied from behind PO moved readily into the

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THE PERMEABILITY OF THE RABBIT CILIARY EPITHELIUM TO HORSERADISH PEROXIDASE IN EXPERIMENTAL UVEITIS

An electron microscopic study

BY

OLAV ØYVIND PEDERSEN

Experimental uveitis has been produced in two groups of albino rabbits by a single *intravitreal injection of antigen* The animals in group I were immunized by injection of 10 mg of human serum albumin, whereas those belonging to group II received 50 mg of the same antigen. To study the blood aqueous barrier to proteins in the ciliary body of these eyes horse *radish peroxidase has been used as a cytochemical tracer by electron* microscopy The tracer was injected intravenously at different time intervals (1 30 min) before enucleation

In group I in regions of inflammatory cell infiltration in the ciliary processes peroxidase reaction product was observed in the lateral intercellular spaces of the superficial epithelium in the internal limiting membrane and in the posterior chamber as evidence of breakdown of the epithelial diffusion barrier In areas showing moderate or no inflammatory cell infiltration the diffusion barrier to peroxidase was found intact although the epithelium displayed marked morphological alterations

In the more heavy inflamed eyes belonging to group II necrosis of the ciliary epithelium and thrombosis of the ciliary vessels were prominent

Key words uveitis experimental – ciliary body – aqueous humor – proteins – peroxidase – rabbits – electron microscopy

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iris hyperaemia was found In some of the eyes posterior synechiae were present but there was no sign of seclusio or occlusio pupillae The lenses appeared normal Moderate blurring of the vitreous body was found In group II the inflammation was more intense A cellular exudate was present in the anterior chamber and the vitreous body was clouded The e eyes were enucleated 3 to 6 days after the onset of the uveitis The control eyes showed no sign of inflammation

Seven rabbits belonging to group I four rabbits in group II and the rabbit whose eyes had not been injected were injected intravenously with 250–500 mg commercial horseradish peroxidase (Sigma type II) dissolved in 5 ml sterile Ringer solution The rabbits belonging to group I were killed 1 2 5 10 15 and 30 min after the injection of peroxidase and the rabbits in group II were killed after 1 5 10 and 30 min The rabbit that had not received antigen was killed 5 min after the injection of the tracer

The rabbits were killed by decapitation and the eyes were immediately enucleated They were opened broadly at the equator and immersed in ice cooled fixative

The preparation of the tissue has been described in detail elsewhere (Pedersen 1973) The tissues were prefixed in 1 % or 2 5 % glutaraldehyde in 0 1 M phosphate buffer The 1 % solution contained 4 % sucrose as well After incubation in tris HCl buffered diaminobenzidine H_2O solution pH 7 6 (Karnovsky 1967) they were postfixed in 1 % OsO_4 in Millonig s phosphate buffer and embedded in Epon 812 Some tissue blocks were stained with uranyl *en bloc* according to Karnovsky (1967)

Ultrathin sections were made with an LKB Ultrotome The sections were stained with alkaline lead or an aqueous solution of uranyl acetate or both Unstained sections were also examined Micrographs were taken with a Siemens Elmiskop 1A

Several blocks from each of the uveitis eyes and at least one from each of the control eyes were examined

As cytochemical control one rabbit belonging to each of the two uveitis groups was injected intravenously with 5 ml Ringer solution without peroxidase and tissues were fixed and processed as for cytochemical reactions

As substrate controls tissues from peroxidase injected animals were incubated in media without either H_2O or the diaminobenzidine

The Ringer injected eyes served as uveitis controls as well as both eyes from the rabbit whose eyes had not been injected

One rabbit belonging to group II was injected intravenously with 1 mg/kg of an antihistamine (dexchlorpheniramine Polaramin *Schering*) and a serotonin antagonist (methysergide Deseril *Sando*) 10 min before the peroxidase injection

Horseradish peroxidase is a relatively small protein (mol wt about 40 000) that can be used as a cytochemical tracer by light and electron microscopy (Graham & Karnovsky 1966) The presence of the enzyme at a particular site is detected by allowing it to act upon diaminobenzidine in the presence of H_2O_2 to yield an electron opaque reaction product in sufficient amount to be visualized

In a previous light microscopic study (Pedersen 1973) peroxidase diffusion was studied in rabbit ciliary bodies in experimental uveitis following a single intravitreal injection of antigen The purpose of the present paper is to present ultrastructural findings from such eyes

The superficial epithelial cells of the ciliary body are girdled by zonulae occludentes blocking the intercellular diffusion of high molecular substances (Shiose 1970 71 Smith 1971, Vegge 1971 72 Shabo & Maxwell 1972 Smith & Rudt 1973) In the present work special attention was paid to studying the permeability of the superficial ciliary epithelium to peroxidase in rabbit eyes suffering from experimental uveitis

Material and Methods

Fourteen healthy adult albino rabbits weighing 2 5–3 kg were used Before experimentation all eyes were found normal determined with slit lamp and ophthalmoscopic examination

Uveitis was produced in the left eyes of thirteen rabbits belonging to two different groups The rabbits in group I (eight animals) received an intravitreal injection of 10 mg of human serum albumin (HSA) dissolved in 0 1 ml Ringer solution The rabbits in group II (five animals) received 50 mg HSA dissolved in the same amount of Ringer solution Into the right eyes of these 13 rabbits 0 1 ml sterile Ringer solution was injected Both eyes of one rabbit were not injected at all

The albumin solution was prepared by dissolving crystallized HSA (AB Kabi Stockholm) in Ringer solution The solution was sterilized through a Millipore filter (pore size 0 2 μm) immediately before use

After topical anaesthesia with 0 2 % oxibuprocaine the solution was injected slowly into the vitreous body about 2 mm anterior to the equator Care was taken to avoid damaging the ciliary body and the lens capsule

In the antigen treated eyes anterior uveitis developed 5 to 10 days afterwards The eyes belonging to group I were enucleated 1 to 6 days after the onset of the uveitis At the time of the enucleation the eyes showed pericorneal injection Aqueous flare cells and fibrin were observed in the anterior chamber Marked


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Results

The processes of the rabbit ciliary body are divided into a posterior group termed ciliary processes and an anterior group termed iridial processes (Kozart 1968) There are morphological differences between the epithelium of these two types of processes as well as evidence of functional differences (Wegner 1961 Kozart 1968) In the present work the histopathological picture and the peroxidase distribution were the same in these two types of processes Hence in the following no distinction is made between them and the processes of the ciliary body are simply termed ciliary processes

Regarding the nomenclature of the epithelial cell layers of the ciliary processes in the following the terms basal and superficial are used as pertaining to the stroma and to the posterior chamber respectively Thus the epithelial cells facing the posterior chamber are called the superficial epithelial cells and those lining the stroma are called the basal epithelial cells

As has been mentioned previously (Pedersen 1973) sections from tissue incubated *en bloc* showed peroxidase reaction product only at the periphery of the blocks Usually the superficial parts of the stroma were stained when tissues were incubated *en bloc* but in some regions the diaminobenzidine had not reached further than to the intercellular space between the epithelial cell layers (Fig 3) When glutaraldehyde fixed frozen sections were incubated the stroma was stained throughout

1 Cytochemical controls

In sections from tissues incubated in media without either H_2O_2 or the diaminobenzidine peroxidase reaction product could not be demonstrated

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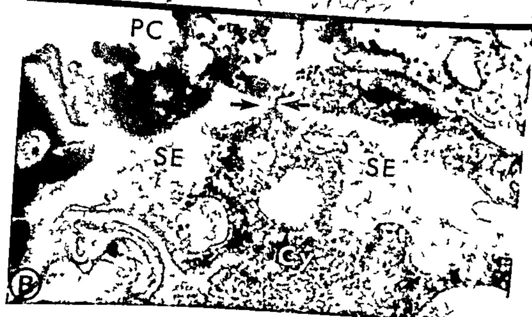
Key to all Figures

Fig 1 micrograph from an eye that had not received antigen Figs 2–4 micrographs from uveitis eyes Posterior chamber (PC) Superficial epithelium (SE) Basal epithelium (BE) Stroma (S) Internal limiting membrane (ilm) Peroxidase reaction product (P)

Fig 1 (opposite page)

Peroxidase distribution in the ciliary epithelium of a control eye Reaction product is present in the stroma between the basal epithelial cells and in the intercellular space between the basal and the superficial epithelial cells No reaction product is seen in the intercellular space (is) between the superficial epithelial cells or in the posterior chamber Peroxidase diffusion has been halted by the basal junctional complex (arrow) of the superficial epithelium This section was not counterstained × 9 300


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There was no peroxidase activity in sections from the animals that had not received the tracer except for endogenous peroxidase activity in the erythrocytes and possibly in some *leucocytes* *These tissues were incubated as for cytochemical reactions*

2 Uveitis controls

The distribution of the peroxidase reaction product was the same in all eyes that had not received antigen Reaction product was seen in the ciliary vessels and the stroma, in the intercellular spaces between the basal epithelial cells and in the intercellular space between the basal and the superficial epithelial cells (Fig 1) The so called *ciliary channels* (Bairati & Orzalesi 1966) were also filled The diffusion of peroxidase was halted by the zonulae occludentes of the basal junctional complexes of the superficial epithelium

Reaction product was observed in some vesicles in the superficial epithelial cells The tracer was not detected in the lateral intercellular space between these cells or in the internal limiting membrane (Fig 1)

Uveitis eyes

Group I In these eyes heavy masses of peroxidase reaction product together with leucocytes and erythrocytes were seen in the posterior chamber In areas of leucocytic infiltration peroxidase reaction product was seen in the intercellular spaces between the superficial epithelial cells as evidence of breakdown of the epithelial diffusion barrier (Fig 2) At these sites the superficial epithelial cells were separated from each other and had more or less lost contact with the basal epithelium In some places vacuoles or cystic dilations were formed between the two epithelial cell layers Now and then the superficial epithelium covering these had ruptured and the cystic dilations were communicating with the posterior chamber (Fig 2)

The cellular infiltration found in these eyes consisted predominantly of mononorphonuclear cells Only scattered granulocytes were found

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Fig 2 (opposite page)

Intercellular leakage of peroxidase through the superficial epithelium A Peroxidase reaction product is seen between the superficial epithelial cells These cells have lost contact with each other at the plane of sectioning Plasma cells (Pl) are seen in the epithelium and in the posterior chamber Lead 11 000 B Part of a cystic dilation (Cy) that has formed between the superficial and the basal epithelial cell layer is seen Peroxidase reaction product is seen in the cystic dilation and in the posterior chamber The cyst [illegible] communicating with the posterior chamber (arrows) The superficial epithelial cells are flattened and have lost their interdigitations Lead [illegible] 000

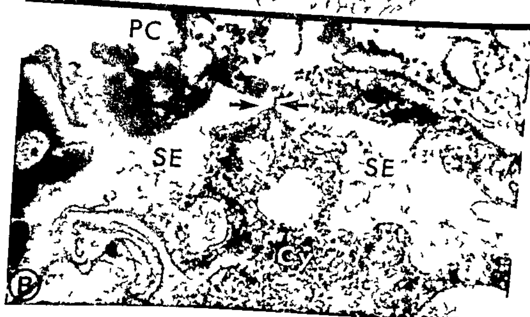

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Fig 4

D lations of the intercellular spaces (is) between the superficial epithelial cells The m rcellular spaces do not conta n perox dase reaction product as evidence of intact z nula cc lu lentes in this region Uranyl *en bloc* 8 400 Inset Area indicated by l uble arr w Diffus on of peroxidase has be n halted at the site indicated by the ar w Th s p r bably represents a z nula occludens Uranyl *en bl c* 43 000

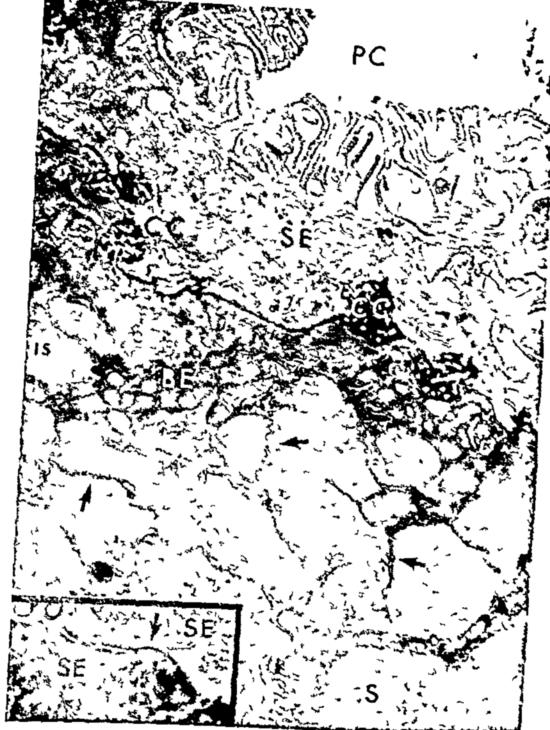
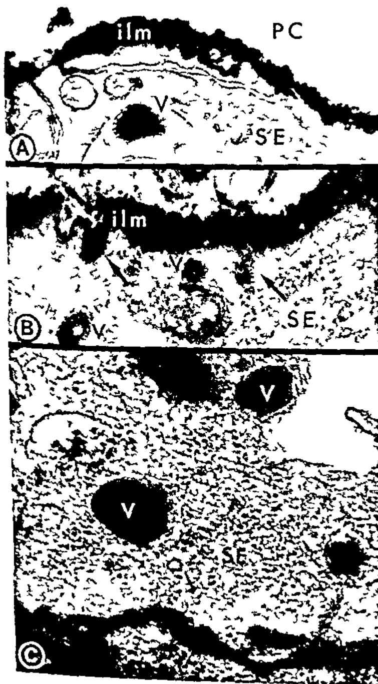


Fig 3

From an area without leucocytic infiltration The basal epithelial cells are partly transformed into thin cytoplasmic strands (arrows) The intercellular space (is) between basal epithelial cells is dilated The intercellular space between the basal and the superficial epithelial cells is not dilated and contains peroxidase reaction product Ciliary channels (CC) Due to *en bloc* incubation reaction product is not present in the stroma in this area Uranyl *en bloc* Lead × 13 ,00 Inset Intact basal junctional complex between superficial epithelial cells *Peroxidase* reaction product is not seen beyond the zonula occludens indicated by the arrow Uranyl *en bloc* Lead × 30 500


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In large areas of the ciliary body, the epithelial diffusion barrier to peroxidase was found intact. In these areas the inflammatory cell infiltration was moderate. The epithelium, however, showed alterations also in these regions. The basal epithelial cells were frequently partly transformed into slender cytoplasmic processes, and the cells were separated by large dilations of the intercellular spaces (Fig. 3). Alterations of the superficial epithelium included loss of interdigitations and widening of the intercellular spaces (Fig. 4). In these areas the neighbouring superficial epithelial cells were united by slender fingerlike projections of the cells.

Shrinkage of the cells and widening of the lateral intercellular spaces of the superficial epithelium are seen in ciliary processes that have been immersed in hypertonic fixatives (Vegge 1972). Most of the eyes in the present study were fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer. The osmolarity of this solution is somewhat hypertonic. It was measured to be 450 mOsmol/l. The eyes of each animal were always treated in the same way. As the control eyes never showed extensive dilations of the intercellular spaces of the basal or the superficial epithelium, this phenomenon is related to the inflammation (compare Figs. 1, 3 and 4). The widening of the intercellular spaces between the epithelial cells in the uveitis eyes is possibly due to increased content of proteins and other substances in the ciliary stroma and the aqueous humor in these eyes.

Vesicles of different sizes, some of which may represent lysosomes, containing peroxidase reaction product, were observed in the superficial epithelial cells (Fig. 5). Now and then, vesicles were seen opening to the intercellular spaces between the superficial epithelial cells beyond the basal junctional complex or to the internal limiting membrane (Fig. 5).

Group II. In these eyes the leucocytic infiltration in the ciliary body was intense, and granulocytes were relatively more abundant than in the eyes belonging to group I. Thrombosis of the ciliary vessels and necrosis of the epithelium were prominent (Fig. 6). Now and then peroxidase reaction product was seen in the cytoplasm of the epithelial cells. The whole cell, except sometimes the nuclei and the mitochondria, was then darkly stained (Fig. 7). This is interpreted as evidence of rupture of the cell membrane prior to the fixation. Such cells were observed in all eyes belonging to group II and in one of the eyes belonging to group I. This eye was enucleated 1 day after onset of the

Fig. 5 (opposite page).

Vesicles (V) containing peroxidase reaction product are seen in superficial epithelial cells. Som of these (arrows) are opening to the internal limiting membrane. Note heavy staining of the internal limiting membrane due to content of peroxidase reaction product. Lead. A: × 31,000. B: × 53,000. C: × 53,000.



Fig /
Blackening of the ytoplasm of superficial epithelial cells due to content of peroxidase reacti n product. The nuclei (N) and mitochondria (m) has not been filled with peroxidase Reaction product is not present in the cell at right Group II Lead × 000



Fig. 6

From a uveitis eye belonging to group II. The epithelium (E) is necrotic and contains fibrin (F). Granular deposits of peroxidase reaction product is present in the epithelium and in the posterior chamber. The lumen (LU) of a ciliary vessel is filled with disintegrated cells. Lead. × 9 100

uveitis and 15 min after the peroxidase injection. None of these cells were observed in the control eyes. In cells undergoing necrosis high molecular substances may enter the cell. Thus fibrin has been observed within such cells (David 1970 p. 398).



Fig. 7

Blackening of the cytoplasm of superficial epithelial cells due to content of peroxidase reaction product The nuclei (N) and mitochondria (m) has not been filled with peroxidase Reaction product is not present in the cell at right Group II Lead × 7 000



Fig 6

From a uveitis eye belonging to group II The epithelium (E) is necrotic and contains fibrin (F) Granular deposits of peroxidase reaction product is present in the epithelium and in the posterior chamber The lumen (LU) of a ciliary vessel is filled with des integrated cells Lead × 9 100

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Fig 1

Blackening of the cytoplasm of superficial epithelial cells due to content of peroxidase reaction product The nuclei (N) and mitochondria (m) has not been filled with peroxidase Reaction product is not present in the cell at right Group II Lead × , 000

Commercial horseradish peroxidase can itself induce vascular leakage in certain tissues in some species, probably due to liberation of histamine and serotonin from mast cells These effects of peroxidase can be inhibited by the combined injection of histamine and serotonin antagonists before the peroxidase injection (Cotran & Karnovsky 1967) No observable changes concerning the peroxidase distribution were seen in the eyes of the rabbit that received these antagonists Accordingly, such effects of peroxidase do not seem to be of practical importance in the study of the rabbit blood aqueous barrier in experimental uveitis

Discussion

In the present study breaks in the epithelial diffusion barrier to peroxidase have been demonstrated in the ciliary bodies of rabbits suffering from acute inflammation of the uvea Breaks in this barrier were usually associated with heavy inflammatory cell infiltration This is in accordance with previous light microscopic observations (Pedersen 1973) The diffusion barrier was intact in areas containing few or no leucocytes although marked morphological alterations of the epithelial cells were observed in these areas

Breaks in the epithelial diffusion barrier were seen at sites where the basal junctional complexes of the superficial epithelium were ruptured At these sites inflammatory cells were frequently interposed between the epithelial cells It has been suggested by Segawa & Smelser (1969) that the polymorphonuclear cells rather than the monomorphonuclear ones play an important direct role in the breakdown of the blood aqueous barrier in experimental uveitis In the present work lymphocytes and plasma cells were frequently seen between the epithelial cells in regions where these cells had lost contact with each other Observations indicating intercellular leakage of peroxidase through the superficial epithelium were seen in areas that appeared to be free of granulocytes However in the present work clusters of granulocytes were not observed and accordingly the effects caused by such aggregations can not be evaluated In the intensely inflamed eyes belonging to group II both the cellular infiltration on the whole and the relative proportion of granulocytes were increased Moreover in these eyes widespread thrombosis of the ciliary vessels was found and consequently the epithelial cells were undergoing necrosis due to ischaemia

In several previous electron microscopic studies changes in the ciliary epithelium following paracentesis of the anterior chamber and systemic administration of bacterial endotoxin have been described (Pappas & Smelser 1958 Smelser

& Pei 1965 Bairati & Orzalesi 1966 Kozart 1968 Howes McKay & Aronson 1971 Shiose 1971) These studies revealed striking morphological alterations of the basal (pigmented) epithelium The cells were frequently separated by large dilations of the intercellular spaces Various degrees of separation of the two epithelial cell layers occurred Flattening of the superficial epithelial cells and loss of their interdigitations were noted After paracentesis of the anterior chamber rupture of the basal junctional complexes of the superficial epithelium was not described by Bairati & Orzalesi (1966) or Kozart (1968) In the present work epithelial changes similar to these mentioned above were observed even in areas showing moderate or no cellular infiltration In these areas the diffusion barrier to peroxidase was found intact It seems that intercellular leakage of proteins through the intercellular spaces of the superficial epithelium is not very prominent following paracentesis of the anterior chamber Although evidence of such leakage has been demonstrated by the use of thorotrast as a tracer (Smelser & Pei 1965) the work of Shiose (1971) indicates that this is not prominent. It was noted by Shiose (1971) that on occasion some occluding zonules of the superficial ciliary epithelium were destroyed and allowed passage of peroxidase The iris vessels on the other hand showed rapid leakage of peroxidase through the interendothelial clefts This is in contrast to the findings in experimental uveitis of the present type Here the iris vessels seem less affected than the ciliary epithelium (Pedersen 1974) This is believed to reflect differences in the pathogenesis of the breakdown of the blood aqueous barrier in these conditions Thus a sudden drop in the intraocular pressure by paracentesis probably causes vascular engorgement (Davson 1972) In the iris the resulting increased hydrodynamic pressure difference between the blood and the aqueous humor will act directly on the vessel walls as there is no barrier between the iris stroma and the anterior chamber In the ciliary body on the other hand the epithelial lining is interposed between the blood vessels and the posterior chamber and the ciliary stroma will be able to accumulate protein containing oedema fluid without rupture of the epithelial diffusion barrier In experimental uveitis on the other hand the main inflammatory reaction takes place in the ciliary body It has been shown that intravitreally injected antigen leaves the eye largely via the anterior chamber (Fernando 1960) and as the base of the ciliary body is in the region of the main passage of aqueous humor it is believed that the initial interaction of antigen with the uveal tissue takes place here (Segawa & Smelser 1969)

Systemic administration of bacterial endotoxin produces marked increase of the permeability of the vessels of the anterior ciliary and the iridial processes (Howes McKay & Aronson 1971) It was suggested by these authors that this is due to an extensive local activation of the clotting mechanism Large inter

Commercial horseradish peroxidase can itself induce vascular leakage in certain tissues in some species probably due to liberation of histamine and serotonin from mast cells These effects of peroxidase can be inhibited by the combined injection of histamine and serotonin antagonists before the peroxidase injection (Cotran & Karnovsky 1967) No observable changes concerning the peroxidase distribution were seen in the eyes of the rabbit that received these antagonists Accordingly such effects of peroxidase do not seem to be of practical importance in the study of the rabbit blood aqueous barrier in experimental uveitis

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In several previous electron microscopic studies changes in the ciliary epithelium following paracentesis of the anterior chamber and systemic administration of bacterial endotoxin have been described (Pappas & Smelser 1958 Smelser

Smith & Rudt (1973) observed a prominent staining of the internal limiting membrane of the ciliary epithelium in monkeys 20–30 min after intravenous injection of peroxidase Shiose (1970) Vegge (1971) and Shabo & Maxwell (19,?) on the other hand found that there was little or no evidence that peroxidase was secreted into the posterior chamber Although peroxidase reac tion product could not be demonstrated in the internal limiting membrane or in the posterior chamber in the control eyes in the present study it can not be excluded that such transport may take place to a limited extent in the rabbit However the amount of peroxidase transported within 30 min was not suffic ient to be detected

The pathophysiological mechanisms involved in the breakdown of the blood aqueous barrier during experimental uveitis are certainly very complex From the present study it seems that mechanical rupture of the basal junctional complexes of the superficial epithelium and necrosis of the ciliary epithelium are of importance for the formation of the proteinaceous aqueous humor Such changes of the ciliary epithelium may e.g be due to a mechanical separation of epithelial cells caused by leucocytes that are piercing the epithelium to the effects caused by circulatory disturbances to the effects caused by chemical mediators or lysosomal enzymes either directly on the epithelium or via effects on the ciliary vessels or to a combination of two or more of these effects Further investigations are needed to evaluate the significance of these different effects on the blood aqueous barrier during acute anterior uveitis

Acknowledgements

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epithelial protein containing cysts were observed in these regions Occasionally the superficial epithelium had apparently ruptured

As the ciliary vessels are permeable to peroxidase under physiological conditions, the use of this tracer does not convey new information concerning a possible change of the permeability of these vessels during anterior uveitis. However thrombosis of ciliary vessels was seen in the eyes belonging to group II and disruptions of endothelial junctions were occasionally observed in group I This indicates that circulatory disturbances are of importance in the pathogenesis of the breakdown of the blood aqueous barrier in experimental uveitis. In immunogenic uveitis of severe grade, thrombosis of intraocular vessels has been demonstrated by several authors (Segawa & Smelser 1969 Levine & Ward 1970 Aronson & al 1974) Moreover increased vascular permeability of the ocular vessels has been demonstrated following intravitreal injection of antigen in rabbits immunized by systemic administration of the same antigen (Aronson & al 1971)

Vesicles containing peroxidase have been found in the superficial epithelial cells in the uveitis eyes in the present study Although some of these i e the ones located at the basis of the cells may represent oblique sections of the intercellular space between the basal and the superficial epithelium there is no reason to doubt that the ones located more apically do represent true vesicles Some vesicles appeared to be opening to the internal limiting membrane It can not be determined on the present evidence whether this is an expression of intracellular transport of peroxidase from the basis of the cells, whereby the occluding zonules are bypassed or reabsorption of peroxidase that has reached the posterior chamber by the intercellular pathway However it is assumed that a possible transport is of minor significance compared with intercellular diffusion It is also possible that peroxidase as a foreign protein is treated differently from the serum proteins in this respect

The distribution of the peroxidase reaction product in the ciliary processes in the eyes that had not received antigen is in accordance with previous findings in mice and monkeys (Shiose 1970 71 Smith 1971 Vegge 1971 Shabo & Maxwell 1972 Smith & Rudt 1973) In the rabbit Uusitalo Palkama & Stjernschantz (1973) found the same distribution in the iridial processes In the ciliary processes and the pars plana they found reaction product in the lateral intercellular spaces of the superficial epithelium "in some places This has not been observed in the present study Some controversy exists regarding the significance of the peroxidase containing vesicles found in the superficial epithelial cells Smith (1971) found the tracer in the anterior chamber of mice within 5 min after intravenous injection of peroxidase He suggests that there is a rapid passage of peroxidase through the superficial epithelium by vesicular transport.

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There is a diffusion barrier to proteins located to the endothelium of the iris vessels The endothelial cells are girdled by zonulae occludentes preventing the diffusion of proteins through the interendothelial gaps of these vessels (Shiose 1971 Smith 1971 Vegge 1971 72)

The purpose of the present work was to study this diffusion barrier in rabbit eyes suffering from acute inflammation of the anterior uvea produced by a single intravitreal injection of antigen Horseradish peroxidase has been used as a protein tracer by light and electron microscopy

Material and Methods

Iris tissue was obtained from the same eyes that were used in a previous study of the ciliary epithelium (Pedersen 1974)

Uveitis was produced in the left eyes of 13 healthy adult albino rabbits belonging to two different groups The rabbits in group I (eight animals) were immunized by a single intravitreal injection of 10 mg of human serum albumin (HSA) dissolved in 0 1 ml Ringer solution The rabbits in group II (five animals) received 50 mg HSA dissolved in the same amount of Ringer solution Into the right eyes of these 13 rabbits 0 1 ml sterile Ringer solution was injected Both eyes of one rabbit were not injected at all

In the antigen treated eyes anterior uveitis developed 5 to 10 days afterwards The eyes belonging to group I were enucleated 1 to 6 days after the onset of the uveitis whereas those belonging to group II were enucleated after 3 to 6 days All antigen treated eyes showed a frank uveitis at the time of enucleation The eyes belonging to group II were more intensely inflamed as compared with those belonging to group I In all uveitis eyes aqueous flare cells and fibrin were observed in the anterior chamber and marked iris hyperaemia was observed The control eyes showed no sign of inflammation

Seven rabbits belonging to group I four rabbits in group II and the rabbit whose eyes had not been injected were injected intravenously with 250–500 mg commercial horseradish peroxidase (Sigma type II) dissolved in 5 ml sterile Ringer solution The rabbits were killed at different time intervals from 1 to 30 min after the injection of peroxidase The rabbit that had not received antigen was killed 5 min after the injection of the tracer

The rabbits were killed by decapitation and the eyes were immediately enucleated The tissues were prefixed in 1 % or 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 The 1 % solution contained 4 % sucrose as well The tissues were washed over night in 0 1 M phosphate buffer containing 5 %

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A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE PERMEABILITY OF THE RABBIT IRIS VESSELS TO HORSERADISH PEROXIDASE IN EXPERIMENTAL UVEITIS

BY

OLAV ØYVIND PEDERSEN

Experimental uveitis has been produced in two groups of albino rabbits by a single intravitreal injection of antigen. The animals in group I were immunized by injection of 10 mg of human serum albumin whereas those belonging to group II received 50 mg To study the blood aqueous barrier to proteins in the iris vessels of these eyes horseradish peroxidase has been used as a protein tracer by light and electron microscopy The tracer was injected intravenously at different time intervals (1–80 min) before enucleation

In group I no leakage of peroxidase from the iris vessels was demonstrated in any eyes except one The diffusion of peroxidase was halted by the zonulae occludentes of the vascular endothelium and there was no evidence of vesicular transport of peroxidase through the endothelial cells In the more heavy inflamed eyes belonging to group II a prominent inflammatory cell infiltration was found in the iris In these eyes leakage of peroxidase through the interendothelial clefts of the iris vessels was demonstrated

Key words uveitis experimental – iris – blood vessels – aqueous humor – proteins – peroxidase – rabbits – electron microscopy

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There is a diffusion barrier to proteins located to the endothelium of the iris vessels The endothelial cells are girdled by zonulae occludentes preventing the diffusion of proteins through the interendothelial gaps of these vessels (Shiose 1971 Smith 1971 Vegge 1971 72)

The purpose of the present work was to study this diffusion barrier in rabbit eyes suffering from acute inflammation of the anterior uvea produced by a single intravitreal injection of antigen Horseradish peroxidase has been used as a protein tracer by light and electron microscopy

Material and Methods

Iris tissue was obtained from the same eyes that were used in a previous study of the ciliary epithelium (Pedersen 1974)

Uveitis was produced in the left eyes of 13 healthy adult albino rabbits belonging to two different groups The rabbits in group I (eight animals) were immunized by a single intravitreal injection of 10 mg of human serum albumin (HSA) dissolved in 0 1 ml Ringer solution The rabbits in group II (five animals) received 50 mg HSA dissolved in the same amount of Ringer solution Into the right eyes of these 13 rabbits 0 1 ml sterile Ringer solution was injected Both eyes of one rabbit were not injected at all

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sucrose Thin slices (0 5–1 mm) of iris tissues as well as glutaraldehyde fixed frozen sections of 40 μm in thickness were incubated in tris HCl buffered diaminobenzidine H_2O_2 solution pH 7 6 (Karnovsky 1967) The tissues were post fixed in 1 % OsO_4 in Millonig s phosphate buffer and embedded in Epon 812 Some tissue blocks were stained with uranyl *en bloc* according to Karnovsky (1967)

Semi thin sections for light microscopy were examined either unstained or stained with toluidine blue Several blocks from each of the uveitis eyes and at least one block from each of the control eyes were examined

Ultra thin sections were made with an LKB Ultrotome The sections were stained with alkaline lead or an aqueous solution of uranyl acetate or both Unstained sections were also examined Micrographs were taken with a Siemens Elmiskop 1A

The cytochemical controls as well as the control for histamine and serotonin liberation induced by the peroxidase injection itself were the same as described previously (Pedersen 1974)

The Ringer injected eyes served as uveitis controls as well as both eyes from the rabbit whose eyes had not been injected

Results

1 Control eyes

No leakage of peroxidase from the iris vessels was found in any of the eyes that had not received antigen The lumina of these vessels were blackened by peroxidase reaction product but this was not found outside the vascular endothelium in the iris stroma (Figs 1 & 4) The diffusion of peroxidase was halted by the zonulae occludentes of the vascular endothelium Peroxidase reaction product appeared to be present in a few vesicles within the endothelium These may however represent invaginations of the vascular lumen There was no evidence that peroxidase was transported through the endothelium and liberated on the stromal side within 30 min These observations are in agreement with previous studies (Shiose 1971 Smith 1971 Vegge 1971 Uusitalo Palkama & Stjernschantz 1973)

2. Uveitis eyes

Group I In these eyes the diffusion barrier to peroxidase in the iris vessels was found intact in all eyes but one (Fig 2) The interendothelial spaces were filled

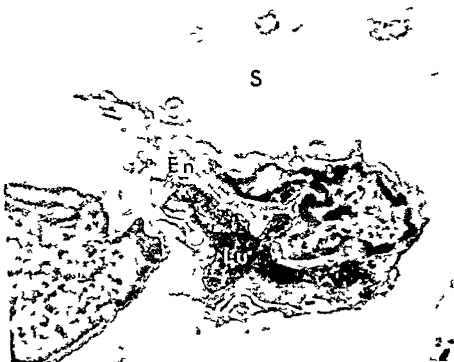


Fig 1

Iris vessel from an eye that had not received antigen The vascular lumen (Lu) is blackened by peroxidase reaction product but this is not found outside the endothelium (En) in the iris stroma (S) Uranyl *en bloc* × 10 000

with peroxidase reaction product as far as to the zonula occludens No reaction product was found in the interendothelial spaces on the stromal side of the junctions (Fig 3) Peroxidase reaction product was found on the anterior and posterior surface of the iris in these eyes and occasionally some could also be detected in the iris stroma It is assumed that the source of this is the ciliary body Thus in the same eyes rapid leakage of peroxidase through the ciliary epithelium has been demonstrated in previous studies (Pedersen 1973 1974) Peroxidase that has reached the anterior chamber will be able to enter the iris stroma via the anterior surface of the iris that has no continuous cellular lining

One of the eyes belonging to this group showed leakage of peroxidase from the iris vessels This eye was enucleated 30 min after the peroxidase injection Accordingly it is possible that this eye in contrast to the other eyes in group I showed leakage of peroxidase from the iris vessels due to a longer time interval

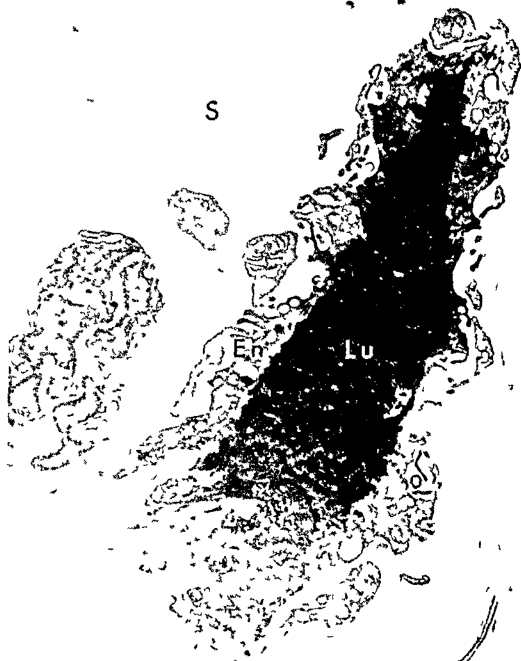


Fig 2
Iris vessel from a uveitis eye belonging to group I The distribution of the peroxidase reaction product is the same as in Fig 1 Vascular lumen (Lu) Endothelium (En) Stroma (S) Uranyl *en bloc* Lead x / 000



Fig 3

Group I Intact diffusion barrier to peroxidase in a venule Peroxidase reaction product is present in the vascular lumen (Lu) and in the interendothelial cleft as far as to the zonula occludens (arrow) No reaction product is seen in the interendothelial cleft beyond the zonula occludens Endothelium (En) Uranyl *en bloc* Lead × 56 000

from the peroxidase injection to the enucleation However the histopathological picture of the iris in this eye was similar to that found in the eyes belonging to group II i e the inflammatory cell infiltration was heavy and this may explain why the iris vessels of this eye showed leakage of peroxidase

The inflammatory cell infiltration in the iris was moderate in the rest of the eyes belonging to group I It consisted almost exclusively of monomorphonuclear cell types

Group II In these eyes observations indicating a rapid leakage of peroxidase through the interendothelial clefts of iris vessels were found By light micro scopy a prominent dark brown staining of the iris stroma was observed (Fig 4)

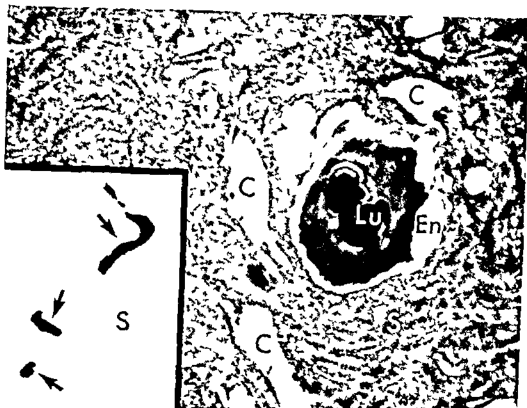


Fig 4

Unstained light micrograph from iris of a uveitis eye belonging to group II Prominent peroxidase activity is present in the vascular lumen (Lu) and in the stroma (S) The vascular endothelium (En) and the cells (C) in the stroma display no significant activity × 1 420 Inset Unstained light micrograph from iris of an eye that had not received antigen Peroxidase activity is present in the vessels only (arrows) Stroma (S) × 690

By electron microscopy peroxidase reaction product was observed in the vascular lumen in the interendothelial clefts throughout their lengths and in the stroma (Fig 5) There was no apparent increase in the number of endothelial vesicles containing peroxidase reaction product as compared with the control eyes Blackening of the interendothelial clefts was observed in the capillaries venules as well as in the small arteries Whether the eyes were enucleated early (1 min) or late (30 min) after the peroxidase injection made no appreciable difference to the localization of peroxidase

Heavy infiltration of monomorphonuclear cells and some granulocytes were found in the iris stroma in these eyes

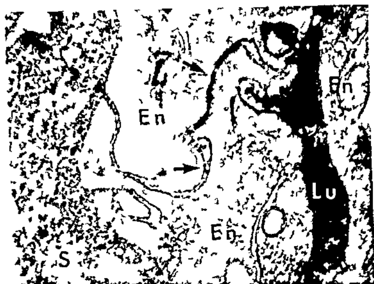


Fig 5

Group II Iris capillary Peroxidase reaction product is present in the vascular lumen (Lu) in the interendothelial cleft (arrows) throughout its length and in the stroma (S) Endothelium (En) Lead × 30 000

Discussion

In the present study breaks in the endothelial diffusion barrier to peroxidase in the iris vessels have been demonstrated in rabbit eyes suffering from intense immunogenic inflammation of the uvea In more moderately inflamed eyes this diffusion barrier was found intact In all these eyes however breaks in the ciliary epithelial diffusion barrier to peroxidase have been demonstrated in previous studies (Pedersen 1973, 74) In experimental uveitis of the present type the main inflammatory reaction takes place in the ciliary body This has been discussed previously (Pedersen 1974) When however the inflammation becomes intense the reaction in the iris will also be prominent. Thus in the present work heavy monomorphonuclear cell infiltration was found in the iris of the eyes belonging to group II and in one of the eyes belonging to group I These same eyes showed leakage of peroxidase from the iris vessels Accordingly it seems that as in the ciliary body the breakdown of the blood aqueous barrier in the iris is in some way related to the inflammatory cell infiltration

Opening of the endothelial junctions of the iris vessels has been demonstrated by several authors Shakib & Cunha Vaz (1966) found large openings after paracentesis and after local application of histamine Sakuragi (1969) demonstrated passage of thorotrast particles through the interendothelial clefts after systemic administration of histamine and Shiose (1971) demonstrated leakage of peroxidase and other tracer substances after paracentesis of the anterior chamber

It was suggested by Shakib & Cunha-Vaz (1966) that the zonulae occludentes of the iris vessels are discontinuous in three dimensions and that these junctions form insecure attachments that have little functional significance Vegge & Ringvold (1969) on the other hand found a zonula occludens between adjacent endothelial cells in human iris vessels wherever sectioning was favourable The same observation was made by Vegge (1972) in a study of the small iris vessels of the vervet monkey Several studies in which peroxidase has been used as a tracer (Shiose 1971 Smith 1971 Vegge 1971 Uusitalo Palkama & Stjernschantz 1973) as well as the present work have shown that this relatively small molecular tracer does not penetrate the interendothelial clefts of iris vessels under physiological conditions It has also been shown that fluorescein (mol wt. of the sodium salt 376) does not penetrate normal rabbit iris vessels (Whitelocke & Eakins 1973) These observations indicate that the tight junctions are continuous in three dimensions and have considerable functional significance in preventing the diffusion of serum proteins to the aqueous humor Moreover the present study indicates that this barrier is not easily broken by an inflammation of the anterior uvea Thus intact diffusion barrier to peroxidase was found in most of the eyes belonging to group I in the present work although iris hyperaemia was observed in these eyes before enucleation

Acknowledgements

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ULTRASTRUCTURAL CHANGES IN THE NORMAL HUMAN LENS CAPSULE FROM BIRTH TO OLD AGE

BY

JOHAN H SELAND

Ten normal lens capsules have been studied by means of transmission electron microscopy A surface layer containing fibres (lamina zonularis) is present on the whole of the ant capsule at birth This layer disappears from the ant pole in the first or second decade of life A laminar architecture of the proper capsule has been confirmed and age dependant loss of lamination has been observed The appearance of electron dense formed elements in the capsule seems to be a specific ageing phenomenon and three types of elements are described Hassall Henle like bodies have been demonstrated in the peripheral part of the capsule

Key words lens capsule – lamina zonularis – capsular laminations – intracapsular formed bodies

The lens capsule is a non cellular and optically clear membrane serving as an encasement for the lens tissue and as an anchorage for the zonular threads It can be traced from the 8th week of gestation and increases in thickness through out life

When stained with aniline blue and examined by light microscopy two

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layers were originally described in the capsule The major and basal part had homogenous staining proper ies and was called capsula propria A distinct superficial tenuous layer was termed the zonular lamella (Berger 1882) Introduction of electron microscopical techniques however have revealed that the capsule consists of fine parallel laminae (Bahr 1954) The first report of nonhomogenicity of the proper capsule was given by Monahan (1954) He described channels in the anterior lens capsule These were later shown to be formed elements (Dark 1961) and some of these elements have been shown to exhibit a definite periodicity (Jakus 1964 Cohen 1965) Dotted and cross striated elements or inclusions in the anterior capsule have later been described by a number of authors (Dark Streteen Jones 1969 Kobaiashi 1969 Ohno 1969 Ogata 1970 Raviola 1971 Hogan et al 1971) The significance of the inclusions has been a matter of dispute Some workers maintain that they represent a part of the zonular apparatus (Raviola 1971 Porte et al 1971) while others are of the opinion that they are unrelated to this (Hogan et al 1971 Dark 1961 Seland 1973) The inclusions have been shown to increase in amount as age advances (Dark et al 1969) and there is also some evidence that they are more abundantly present in fibrillopathia epitheliocapsularis *so called* senile exfoliation or pseudo exfoliation (Bertelsen & Seland 1972) No formed elements have been described in the posterior capsule

There is at the present time little doubt that the lens capsule is produced by

Table I

	Age	Sex	Source
Lens no 1	Newborn	Female	Stillbirth
Lens no 2	Newborn	Female	Stillbirth
Lens no 3	6 yrs	Male	Orbital sarcoma
Lens no 4	1 yrs	Male	Traumatic postscleral rupture
Lens no	5 yrs	Male	Lens extraction prior to keratoprosthesis
Lens no 6	6 yrs	Male	Corneal donor
Lens no	68 yrs	Female	Corneal donor
Lens no 8	69 yrs	Female	Corneal donor
Lens no 9	74 yrs	Male	Corneal donor
Lens no 10	54 yrs	Female	Corneal donor

the lens epithelium. This has been demonstrated *in vivo* (Young & Ocumpaugh 1966) and *in vitro* (von Sallman, Grimes & Alberth 1969). The postnatal growth of the capsular substance has been investigated by Fisher & Pettet (1972) who concluded that only the anterior capsule increases with age. The increase was greatest at the insertion of the anterior zonular threads.

Physical properties of the capsule like elasticity and breakingpoint force are also lowered with increasing age (Fisher 1969).

The lens capsule is a unique basement membrane reflecting the activity of the epithelial cells. Reports of changes thought to be specific of certain pathological conditions like fibrillopathia epitheliocapsularis, so called senile exfoliation or pseudoexfoliation, have been described by several authors (Bertelsen, Drablos & Flood 1964, Ashton et al. 1965). In order to draw conclusions from investigations of capsules from pathological lenses, it was decided to examine closely the changes occurring in normal lens capsules from birth to old age.

Material and Methods

Ten normal lenses have been studied. Their age and source are tabulated in Table 1. The donor eyes were removed within 3 hours after death and kept at + 3° C until the cornea had been utilized. The lenses were removed not later than 18 hours after death and fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.3) for 1½ to 2 hours. The lenses were transsected and the nuclear fibres were removed. Only the superficial cortex and the capsule were processed further. The two halves of the lenses were postfixed in osmium tetraoxyd, dehydrated in increasing concentrations of ethanol and embedded in Epon 812 (Luft 1961). After polymerisation radial slices were cut and these slices were again sectioned into eight subsections (Fig. 1). Each subsection was orientated and reembedded in Epon 812, cut in a Reichert of LKB ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope.

Definitions

In the present investigation the anterior capsule is defined as the capsule covering the epithelial cells. The bow area constitutes the peripheral limit. By this definition, the equatorial region is a part of the anterior capsule. The anterior capsule is divided into five zones (Fig. 1). Zone 1 represents the central anterior area and zone 2 an intermediate ring. The peripheral anterior zone

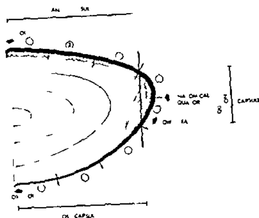


Fig 1
Subdivision of the lens capsule into zones

3 corresponds roughly to the germinative zone Zone 4 is the anterior part of the equatorial area and zone 5 is the posterior part of the equator and the bow area

The posterior capsule rests on lenticular fibres It is limited by the bow area This part of the capsule has been divided into a peripheral zone (6) an intermediate zone (7) and a central zone (8) Each zone is 1–1 5 mm wide

The anterior capsule

The thickness of the neonatal anterior capsule was uniform and measured about 4 μm (Fig 2 A) Tiny threadlike fibres were seen in the most superficial part At the anterior pole they were scanty and each measured about 100 Å in thickness and were up to 1.500 Å long (Fig 2 B) The amount of surface fibres increased greatly towards the equatorial region and in zones 3 and 4 they changed into a typical wavy configuration with a more linear orientation of the surface strands This surface fibre layer penetrated about 0 6 μm into the capsular substance

The main part of the anterior capsule consisted of numerous parallel laminae which were repeated regularly at about 400 Å intervals near the cells This distance decreased towards the capsular surface especially in zones 3–4 Each lamina seemed to consist of myriads of *very fine fibrils* with no signs of periodicity or cross striation No electron dense elements could be observed in the capsule

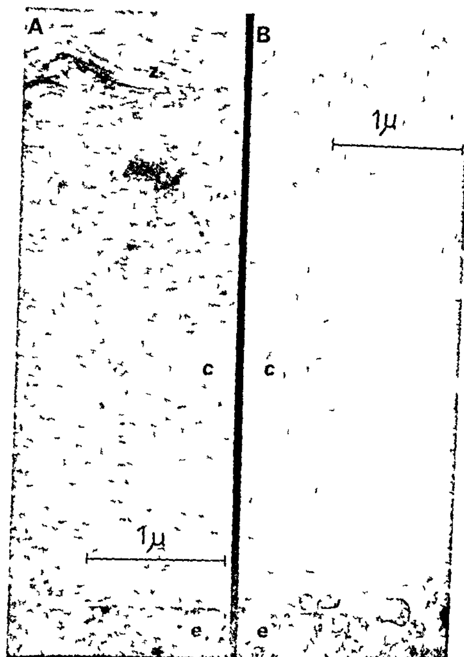


Fig 2

A Newborn female zone 4 Note the laminated capsule (*c*) where the distance between the lamina seem to decrease towards the surface z – zonular threads e – epithelial cell (× 30 000)

B Newborn female zone 1 The lens capsule (c) consists of numerous laminae Note the surface fibrils e – epithelial cell (× 30 000)

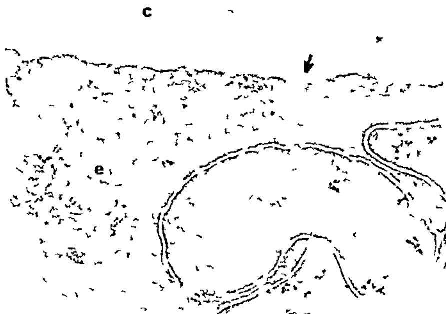


Fig 3

69 yrs female zone 2 Note absence of laminae The capsule (c) consists of myriads of randomly orientated fibrils The diffuse surface membrane areas (arrow) are probably due to tangential sectioning e – epithelial cell (X 42 000)

At the epithelio capsular border an electron lucent zone was quite evident This zone followed the cellular membrane contour and was of the same electron density as the electron lucent zone observed between the capsular laminae In the epithelio capsular electron lucent zone one could often find round electron dense areas particularly at the intercellular junctions (Fig 2 A) They probably represent slender cellular processes

By the age of 6 the thickness of the anterior capsule had almost doubled (μm) and was fairly uniform The threadlike surface elements could still be seen at the anterior pole but they seemed to be fewer in amount. In the anterior proper capsule the laminations were very prominent and the distance between successive laminae was 700 to 800 Å decreasing towards the surface No electron dense intracapsular formed elements could be traced at this stage

The electron-lucent zone at the capsulo epithelial border was prominent in all areas

As age increased one noted the following changes in the anterior capsule

Increase in capsular thickness

Loss of surface fibres at the anterior central parts

Loss of lamination

Appearance of electron dense formed elements (inclusions)

The thickness of the whole anterior capsule increased gradually and in zone 3 it reached 30 μm in Lens 9 Centrally it usually measured 20 to 25 μm in old age No attempt has been made to try and depict the exact changes in the thickness as this would be subjected to many process artifacts An excellent study on fresh lens capsules has recently been published Fisher & Pettet (1972)

No surface fibres were seen at the anterior pole from 17 years and onwards and the texture of the naked area was very loose (Fig 8 A) In the peripheral zones (3 4 5) however the thickness of the zonular layer seemed to increase with age

The loss of laminations in the anterior capsule occurred usually after the age of 50 and was first evident in the basal parts of the anterior polar region subsequently it included more peripheral parts of the anterior capsule One capsule showed no laminations in segments 1 and 2 at the age of 60 (L 7) while another at that age still had laminations discernable in area 2 (L 8) The loss of lamination seemed to be due to disappearance of the electron lucent zones between the laminae leaving a more homogeneous capsular structure consisting of myriads of randomly orientated tiny filaments in an amorphous ground substance The size of these filaments was difficult to judge but the length of each could only be traced for 100 to 300 Å and the thickness seemed to be between 50 and 70 Å (Fig 3)

At the epithelio capsular junction the electron lucent zone may partially disappear at old age One may observe an apparent direct continuity between the intracellular content and the lens capsule (Fig 3) This is most probably an artifact due to oblique sections through the surface membrane Parallel strands were also seen intracellularly near the cell membrane together with a mesh work structure The strands had a typical repetitive pattern of about 160 Å units The thickness of the strands was about 200 Å (Fig 4)

Electron dense-formed elements (inclusions) were seen in the proper capsule at the age of 17 and onwards They first appeared in the anterior equatorial and the anterior peripheral regions (zones 4–3) and were noted maximally 6 μm from the capsulo epithelial border At times they could be seen indenting

A

c

01μ

B

160 Å

m

01μ

C

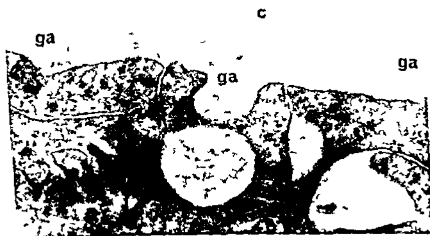


Fig 4

A [illegible] yrs female zone 4 Note the intracellular parallel strands with 160 Å repetitive pattern (arrows) The criss cross meshwork (m) is probably part of the same structure (× 40 000)

B Enlarged square of Fig 4 A demonstrating 160 Å periodicity (× 160 000)

C [illegible] yrs male zone 4 Granular aggregates of electron dense material (ga) near the cell membrane Some material seems to be in the process of being secreted from the cell c – lens capsule (× 40 000)

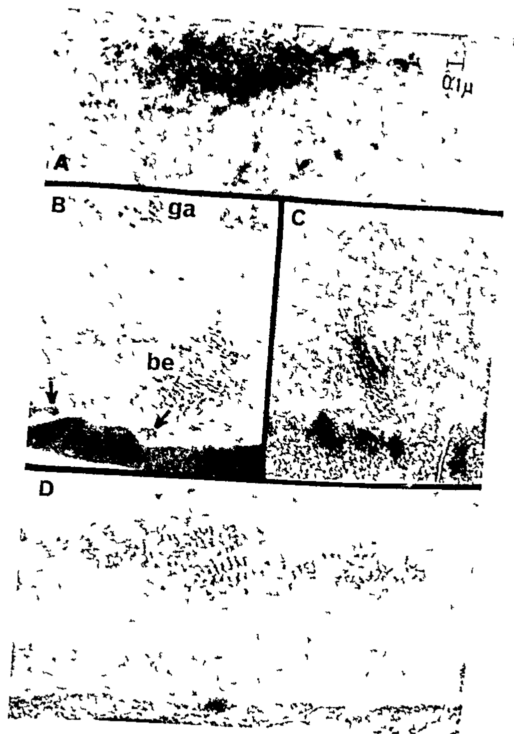


Fig 5

A 69 yrs female zone 4 Granular aggregate of electron dense dots located approx 2 μm from the cell surface Note the tendency to regular banding in the central part Single dots can also be seen (× 32 000)

or even partially enclosed in the epithelial cell (Fig 4 C) The amount of formed elements increased with increasing age and they were localized particularly to zones 3 4 and 5 They could however also be found quite near the anterior pole in the superficial capsule at old age They then appeared as a dotted line (Fig 8 A) Occasionally aggregates have also been found in the peripheral part of the posterior capsule (zone 6) Three types of formed electron dense elements may morphologically be distinguished

The commonest type of electron dense element was elongated aggregates consisting of round randomly placed dots with the diameter 70–100 Å (Fig 5 A) The long axis of such a granular aggregate measured up to 3 5 μ in length and was usually parallel to the capsular surface Single dots may also occur outside the aggregates This type could be found in all capsules from persons older than 1? years In old capsules they could be found at any depth A tangential section through the equatorial capsule revealed that these granular inclusions are part of a system of curved and interconnected electron dense elements (Fig 6)

The second type consisted of electron dense structures which were round or slightly elongated and placed regularly like beads on several parallel strings The size of the structures varied between 120 Å and 300 Å and the distances between the bead strings are about 250 Å This type was always seen near the epithelial cell membrane (Fig 5 B)

The last type seems to be a further development of the latter type 2 where the regularity was enhanced to such an extent that the beads formed transverse bands (Figs 5 C D Fig 7) The distance between the bands was 480 to 520 Å Each element consisted of multiple parallel strands which were about 120 Å thick The long axis of the cross striated fibres was parallel to the epithelial cell surface as a rule Only very occasionally could cross striated fibril bundles be seen orientated perpendicularly to the cell surface (Fig 5 C) The amount of this type seems to increase with increasing age and was usually

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Fig 5 continued (opposite page)

B 4 yrs male zone 3 beadstring element (be) near the cellular border Some beads are circular and others elongated The size of the elements varies between 1 0–300 Å Note the round electron dense elements in the electron lucent cellular border zone (arrows) ga granular aggregates (X 32 000)

C (S y s female zone 3 Epitheliocapsular border Banded fibres with their long axes orientated approx 40 to the cell surface. The distance between the bands is 5?0 Å (X 3? 000)

D (9 yrs female zone 4 Band formed element consisting of several parallel fibres The distance between the bands is 450–5 0 Å (X 3_ 000)

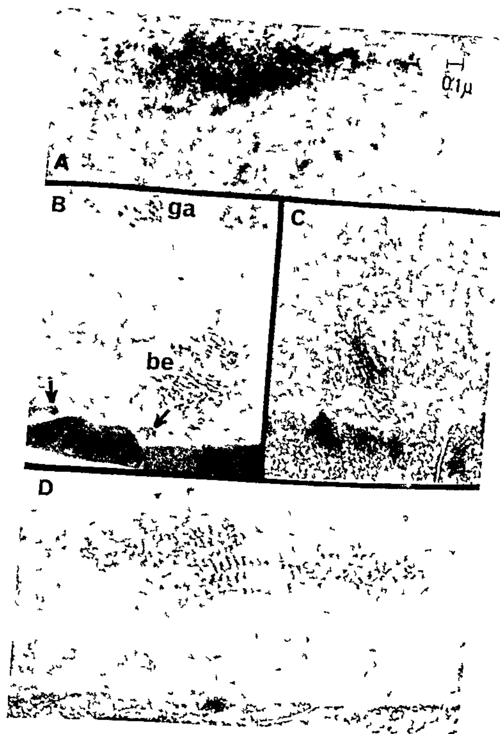


Fig
A 69 yrs female zone 4 Granular aggregate of electron dense dots located approx 2 μm from the cell surface Note the tendency to regular banding in the central part Single dots can also be seen (× 32 000)



Fig 6

63 yrs zone 4 Tangential section through the capsule The granular aggregates form a system of partially interconnected curves (X 44 000)

found in the inner 1/3 of the capsule No elements of this type were found in lenses 1–5

In old age intermediate or mixed forms were not uncommon (Fig 5 A)

Posterior capsule

The changes in the three posterior zones were almost identical

At birth the thickness of the posterior capsule in the polar region was about 3 5 μ Laminations could be observed in the capsule (Fig 8 B) but no fibres could be seen on the surface and no formed bodies were seen within the capsule An electron lucent zone at the capsulo fibrous junction was not a constant feature Certain phenomena specific to the posterior capsule however could be observed At the fibro capsular junction the fibres separate to form open spaces or lacuna which may be empty or partially filled with electron dense material The electron dense material may have a circular or an irregular outline The irregular "granuloma like material was often in contact with the inner capsular surface and if the capsule was torn away from the fibres the material could be seen to adhere to it

By the age of 6 the thickness was still measured to be 3 5 μ at the posterior pole but laminations seemed to have disappeared and no intralacunar granulomata were observed In some sections single threadlike fibres could be seen on the surface but they did not penetrate into the capsule The lens capsule itself had a homogeneous structure and no formed elements were seen

With progressing age only a very slight increase in thickness occurred at the posterior pole (25 years – 4 μ) and the structure seems to be unchanged

From the age of 17 a specific phenomenon could be noted in sector 6 near the bow area One found villi of cell fibres in the capsular substance This phenomenon was accentuated in old capsules where long extensions penetrated up to 2/3 of the total capsule (Fig 9) The extensions or microvilli seemed to consist of material of similar electron density to that found in lenticular fibres This phenomenon resembles the Hassall Henle bodies or corneal warts found in Descemet s membrane

Discussion

It is quite evident that the nature of the examined tissues is not ideal for cellular electron microscopic examinations as some hours may elapse from when the eye is removed from the donor until the fixation process can start

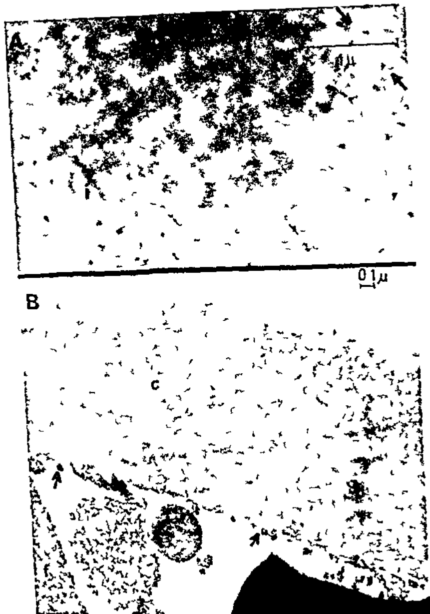


Fig 8

A 84 y male zone — Lens capsule surface denoted by arrows. Note the streak like granular inclusions (i) (X ~5 000)

B Newborn female zone Lam nated post capsule (c) Adhering to the inner surface of the apsule ele tron dense granul mata are seen (arrows) No surface fibres are present Lens fibre (f) (X 32 000)



Fig

74 yrs male zone 3 Band formed elements (b) and granular aggregates (ga) in a lens capsule (c) which reveals some tendency to lamination Periodicity of the cross striations – 500 Å e – epithelial cell (× 50 000)

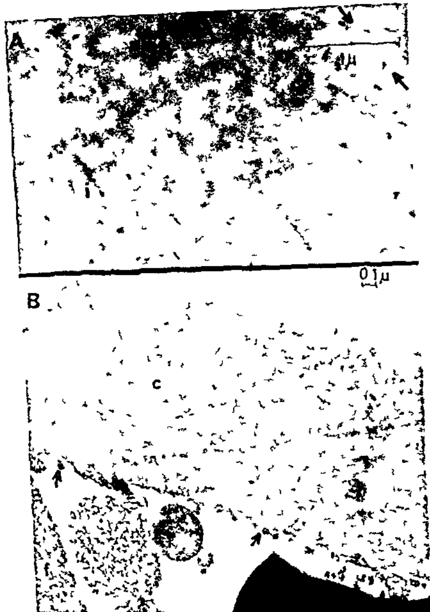


Fig 8

A 54 yrs male zone Lens capsule surface denoted by arrows Note the streak like granular inclusions (i) (X ..000)

B Newborn female zone / Laminated post capsule (c) Adhering to the inner surface of the capsule electron dense "granulomata" are seen (arrows) No surface fibres are present Lens fibre (f) (X 3 000)

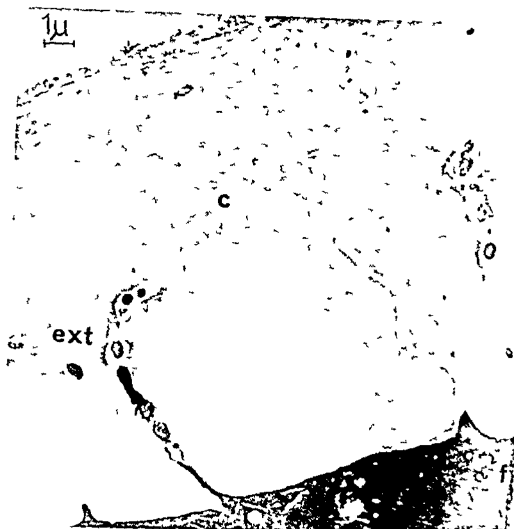


Fig 9
84 yrs male zone 6 Lens fibre extensions (ext) into the posterior lens capsule (c) f – lens fibre (× 6 600)

Basal membranes are not however affected to the same extent by post mortem changes and it is therefore unlikely that all the present observations of the lens capsule are due to artifacts The neonatal lens tissue is covered by an almost uniformly thick laminated capsule On its anterior surface there are tiny threadlike fibrils penetrating about 6 μ into the capsule This fibrillar surface layer represents the anterior zonular attachment and thus confirms the presence of an anterior zonular lamella (Berger 1882) The fibrillar surface layer disappears or retracts from the anterior pole between the age of 6 and 1[illegible]

Threadlike fibres have also been found lining the posterior capsule But apart from the posterior zonular attachment in zone 6 these fibres are not a constant feature and do not seem to penetrate into the capsular substance or to be related to age or location These surface threads probably represent the hyalido capsular membrane

The presence of capsular laminations depends on the age of the lens It is lost first at the posterior capsule before the age of 6 and in adult age (56 years) it has started disappearing from the anterior polar region The lamination persists in the equatorial and pre equatorial regions corresponding to the metabolically most active part of the lens tissue (*zona germinativa*) These findings confirm an earlier observation (Ogata 1971) where the laminar structure was found to be absent in the basal parts of capsules in cataractous lenses

Lamination seems to be a genuine sign of a relatively active capsular production The presence of laminae may also have some bearing on the tensile strength of the capsule as young capsules have a breaking strength 3 times that of old capsules (Fisher 1969) The loss of a laminar structure may be due to a combination of factors including stretching (growth expansion of he lens) a low rate of neogeneses and probably unknown biochemical changes Stretching and thinning of the original capsule may also account for the streaklike appearances of the superficially formed bodies in the anterior capsule at old age (Fig 8 A) It is also a common experience that a rent in the capsule results in an outward rolling of the capsule indicating a greater contractibility of the superficial layer than the deep

The phenomenon of loss of laminations has also been described in Descemet s membrane The corneal endothelium produces a laminar membrane during intrauterine life (Wulle 19,?) The laminations are later replaced by a homogeneous structure as age progresses

The uniformity of the neonatal posterior and anterior lens capsule suggests either that the prenatal lens fibres are able to synthesize capsular material at the same rate as the epithelial cells or alternatively that the epithelial cells prenatally secrete capsular material not only towards their own capsule but also towards the interior of the lens The production of posterior capsular substance must gradually subside to a mere maintenance of a constant thickness as the lens grows

In this investigation no electron den e formed capsular elements were found in the neonatal capsule. They were first encountered at the age of 1, The most superficially formed elements in this lens were seen about 6 μ from the epithelial surface According to our knowledge about capsular growth these inclusions must have been produced by the epithelial cells about the age of

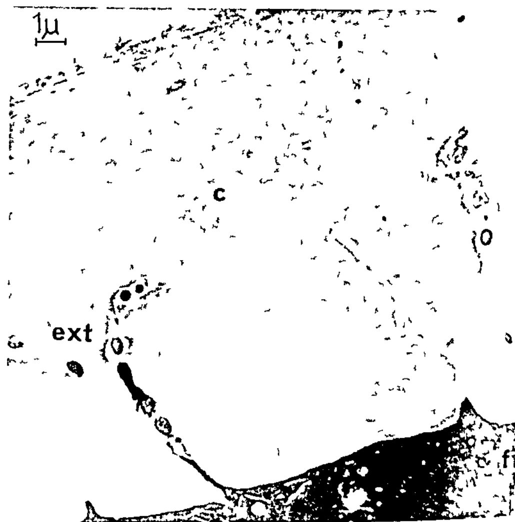


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carried to the surface by means of apposition of capsular material from the inside and a slow removal from the outside An upset in the balance between these two processes can explain the capsular thinning in old age The thickness also depends on the longitudinal stretching caused by increase in lens volume (Fisher & Pettet 19+2)

The presence of Hassall Henle like bodies in the periphery of the lens seems to have no direct bearing on the formed elements The microvilli may however constitute a point of easy access for noxius substances to the lenticular tissue from the aqueous humour

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The amount of inclusions increases greatly with age The type of inclusions are also to some extent age dependant In the posterior capsule no intracapsular formed bodies are found except in the most peripheral parts of old capsules

All these observations seem to support the theory that the intracapsular formed bodies are excretion products from the metabolically most active epithelial cells and not a part of the zonular apparatus

The nature of the inclusions is very uncertain Cohen (1965) has suggested an atypical collagen on the basis of the morphology of the cross striations The periodicity of these striations differs in the literature 500-600 Å (Cohen 1965) 500 Å (Dark Streeten & Jones 1969) 490 Å (Raviola 1971) 600-700 Å (Ogata 1970) The present investigation shows a remarkably constant repetition periodicity of 480 to 520 Å These figures depend however on many external factors in the preparation process and different investigations cannot be directly compared

Different staining methods have suggested a composition related to the zonular threads (Dark 1961) According to Wollensak (1965) these threads do not consist of collagen as judged from amino acid analysis A collagenous antigenic factor in the lens capsule has been isolated but this factor is also present in animals which have no capsular formed bodies Other atypical collagens ("curly collagen' or lattice collagen) have been identified in the eye (Rohen & Lutjen Drecoll 1971) but this seems to be a distinct morphological entity Collagen has also been identified in the lens in a case of congenital cataract (Hendkin & Prose 1967)

The formation of collagen from tropocollagen is considered to be an extracellular process Although the electron dense formed bodies have been found extracellulary cross striated structures have also been demonstrated intracellulary (Fig 5) Even though the majority of investigations suggest a collagenous nature the present evidence is by no means overwhelming Further biochemical histochemical and immunological investigations are required to identify the substance or group of substance involved

Some workers have noted a thinning of the anterior capsule in old age (Salzmann 1912) and this implies a loss of capsular substance This phenomenon is demonstrated in an indirect way by the location of the formed elements The inclusions are found approaching the capsular surface as age advances and at old age it can sometimes be demonstrated in the vicinity of the zonular attachment Assuming that the inclusions are produced by the epithelial cells and have no means of selfpropulsion they must have been

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THE INTRAVITREAL USE OF CARBENICILLIN (GEOPEN) FOR TREATMENT OF PSEUDOMONAS ENDOPHTHALMITIS

BY

ALAN G. SCHENK, GHOLAM A. PEYMAN and JUDITH T. PAQUE

We found that intravitreally injected carbenicillin (Geopen®) in a dose of ? 0 mg or lower is nontoxic to all intraocular structures. A 3.0 mg dose produces levels bactericidal for many strains of *Pseudomonas aeruginosa* up to 4 hours. Experimentally induced Pseudomonas endophthalmitis was successfully treated 10 hours after inoculation by a single intravitreal injection of 5.0 mg of carbenicillin. Eyes treated intravitreally at 24 hours and the eyes of rabbits treated intramuscularly with carbenicillin all progressed to phthisis bulbi. The success of intravitreal treatment is inversely related to the time lapse between inoculation and therapy.

Key words: intravitreal injection – carbenicillin – antibiotics – endophthalmitis

Bacterial infection of the vitreous may result from penetrating wounds, from ophthalmic surgery, as a consequence of bacterial spread from contiguous extraocular structures, or as a metastatic sequel, bloodborne from a focus elsewhere

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We found that intravitreally injected carbenicillin (Geopen®) in a dose of 2 0 mg or lower is nontoxic to all intraocular structures A 3 0 mg dose produces levels bactericidal for many strains of *Pseudomonas aeruginosa* up to 24 hours Experimentally induced Pseudomonas endophthalmitis was successfully treated 10 hours after inoculation by a single intravitreal injection of 5 0 mg of carbenicillin Eyes treated intravitreally at 24 hours and the eyes of rabbits treated intramuscularly with carbenicillin all progressed to phthisis bulbi The success of intravitreal treatment is inversely related to the time lapse between inoculation and therapy

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in the body However it remains an ophthalmic catastrophe since present day treatment methods are usually ineffective

The refractory nature of vitreal infections results from the low and inconsistent levels of antimicrobial drugs attainable in the vitreous after systemic and topical therapy Published studies indicate that while many antibodies including carbenicillin (Geopen®) reach effective levels in the aqueous after subconjunctival injection the penetration into the posterior segment is negligible for all drugs tested thus far (Sery Satya & Leopold 1957 Furgiuele Sery & Leopold 1960 Deuer & Maas 1962 Furgiuele 1964 Kurose Sery & Leopold 1964, Green & Leopold 1965 Records 1966 Fugiuele 1967 Records & Ellis 1967 Furgiuele 1970 Golden & Coppel 1970 Coles et al 1971 Boyle et al 1972) The problem has therefore been that of achieving adequate concentration in the vitreous of an antimicrobial effective against the invading organisms Direct intravitreal injection was first accomplished using penicillin (von Sallmann Meyer & Di Grandi 1944 Leopold 1945 Mann 1946 Duguid et al 1947 von Sallmann 1948 Maylath & Leopold 1955) The intravitreal technique was abandoned for almost two decades however we recently used it in a comprehensive study (Peyman et al 1974 Daily Peyman & Fishman 1973 May et al 1974 Schenk & Peyman 1974) part of which is reported here

Materials and Methods

Carbenicillin was chosen for investigation for two reasons 1) it is significantly active against most strains of *Pseudomonas aeruginosa* an organism that is frequently implicated in postoperative endophthalmitis (Allen & Magiaracine 1964) and 2) the low toxicity of a penicillin allows for intraocular injection of a relatively high dose without the tissue damage produced by many other antibiotics (von Sallmann Meyer & Di Grandi 1944 Peyman et al 1974)

Toxicity

Carbenicillin was injected into the vitreous of 40 albino rabbits each weighing approximately 2 to 3 kg and having vitreouses ranging from 1 2 cc to 1 4 cc Each rabbit was first anesthetized with 50 mg sodium pentobarbital and given 0 12 mg of atropine sulfate intramuscularly to inhibit bronchospasm The pupils were dilated at this time with 1 % cyclopentolate and opthaine drops were applied topically Grasping the superior rectus muscle with a toothed forceps

the following doses of carbenicillin each consisting of 0 1 ml of a sterile water dilution were injected into the vitreous of the right eyes 20 mg 15 mg 10 mg 9 mg 8 mg 7 mg 6 mg 5 mg 3 mg 1 mg and 0.5 mg An anterior chamber paracentesis was performed after safe entry of the needle into the vitreous and prior to injection of the drug The left eyes each injected with 0 1 cc of normal saline solution served as controls Subsequently the eyes were dilated with atropine sulfate and examined daily by direct ophthalmoscopy for 2 weeks and then biweekly for 6 weeks Electroretinograms and applanation tonometry were performed The animals were then sacrificed the eyes were enucleated and fixed in neutral buffered formaldehyde glutaraldehyde solution dehydrated with alcohol embedded in paraffin and sectioned Sections were fixed with hematoxylin and eosin and examined by light microscopy

Clearance

Six albino rabbits were used to study the rate of clearance of carbenicillin from the eye after intravitreal injection Each rabbit was anesthetized as previously described and 3 0 mg of carbenicillin in a 0 1 cc volume of sterile water was injected into the vitreous of both eyes No anterior chamber paracentesis was performed At timed intervals of 0 2 6 16 24 and 45 hours an animal was sacrificed the eyes were enucleated and immediately frozen in liquid nitrogen The frozen vitreouses were dissected away from the eye tissue and assayed the same day for carbenicillin levels by the plate inhibition method using the organism *Sarcina lutea* in which zones of inhibition on each plate were measured and compared to those produced by a known carbenicillin concentration

Treatment of experimentally induced endophthalmitis

A cotton tipped applicator was twirled on a cultured lawn of *Pseudomonas aeruginosa* obtained from a human infection was dipped into 10 cc of brain heart infusion broth and was incubated for 22 hours at 37 C. Previously we had found by serial dilutions with normal saline solution and repeatedly plating out the dilutions that 0 1 cc of a 10^{6} dilution of the cultured broth contained approximately 500 to 1 000 viable organisms The Pseudomonas used for this experiment was found to be moderately sensitive to carbenicillin by the Kirby Bauer method

Each rabbit was anesthetized as previously described and the Pseudomonas inoculum was injected into the vitreous of both eyes of 15 albino rabbits Eyes were treated at graduated time intervals with 5 mg of carbenicillin diluted with sterile water to a volume of 0 1 ml Six eyes (three rabbits) were treated at 3

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Fig 1

Histologic examination at 6 weeks in a rabbit eye injected with 15 0 mg of carbenicillin showed areas of retinal degeneration (Hematoxylin eosin, × 200)



Fig

Hi t l g c f nd ngs at 6 weeks in a rabbit eye injected intravitreally with >0 mg of carben cill n. Note normal retinal structure. (Hematoxyl n eos n, × 200)

hours, six eyes at 6 hours six eyes at 10 hours six eyes at 24 hours and two eyes at 36 hours The four remaining eyes (two rabbits) were not treated intra vitreally with carbenicillin These two rabbits erving as a control group received 800 mg of carbenicillin in a divided dose intramuscularly for 8 days beginning 3 hours after inoculation

Results

Toxicity

Two to four hours after injection there was grossly mild evidence of trauma which included petechiae in the sclera mild conjunctival injection and in several instances fibrinous reaction at the paracentesis site in the anterior chamber All these signs had cleared completely by 48 hours after The only eyes developing cataracts were those injected with 10 0 mg or more of carbenicillin and the cataracts consisted of diffuse speckling on the posterior lens in the eyes receiving 10 0 mg 15 0 mg and 20 0 mg of carbenicillin These cataracts cleared within 4 to 5 weeks Ophthalmoscopically no retinal abnormalities were detected even in the eyes that later histologically showed a retina with partial degeneration However retinal detachments with or without retinal tears were produced with some regularity in the eyes injected with 15 0 mg and 20 0 mg of carbenicillin

Histology

Of the 40 eyes injected 28 of these were fixed for sectioning and hematoxylin and eosin staining Toxicity when manifested consisted chiefly in retinal destruction of the same magnitude as that demonstrated with the higher doses of gentamicin and lincomycin (Daily Peyman & Fishman 1973 Peyman et al 1974) After injection of the 15 mg and 20 mg doses the retina in several areas showed disorganization of the nuclear layers and retinal attenuation with layer disorganization in other areas (Fig 1) In the dose range of 8 to 10 mg several eyes showed toxicity consisting of circumscribed areas of thinning of the nuclear layers and disappearance of the outer segments One eye receiving 9 mg and one receiving 10 mg each showed a large area of degeneration of all layers of the retina Other eyes in this range showed no toxicity at all Using 7 0 mg or a lower dose no retinal toxicity or toxicity of the other ocular tissues was



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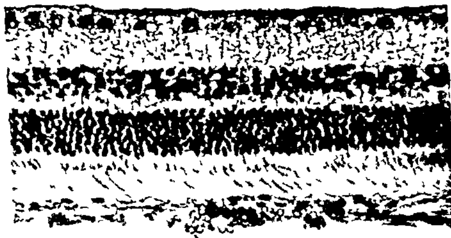


Fig 2.

Histologic findings at 6 weeks in a rabbit eye injected intravitreally with 50 mg of carbenicillin. Note normal retinal structure (Hematoxylin eosin, × 200)

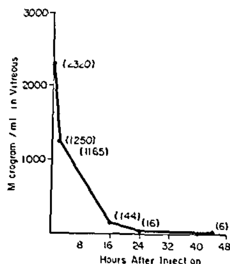


Fig 3
Clearance from the vitreous of a 3 0 mg dose of carbenicillin injected intravitreally into a noninflamed rabbit eye

apparent (Fig 2) One 5 0 mg eye was subjected to electroretinography and applanation tonometry both before and after carbenicillin injection and was found to have a normal ERG and a normal intraocular pressure

Clearance

Bacterial levels of carbenicillin After injecting a 3 0 mg dose of carbenicillin into the vitreous levels bactericidal for most strains of Pseudomonas remained in the vitreous longer than 16 hours and levels effective against many strains of Pseudomonas remained in the vitreous for up to 24 hours (Fig 3) A concentration of 50 μg/ml carbenicillin is considered necessary to inhibit the growth of most strains of *Pseudomonas aeruginosa* (Boyle Gwan Zinn & Leopold 1972)

Treatment

All the rabbit eyes inoculated with Pseudomonas became noticeably inflamed within 3 hours showing a slight yellow conjunctival discharge By 6 to 8 hours after inoculation the eyes demonstrated conjunctival edema mild iritis and a minimal central vitreal clouding By 10 to 12 hours there was increasing vitreal cloudiness and opacification By 24 hours there was severe conjunctivitis

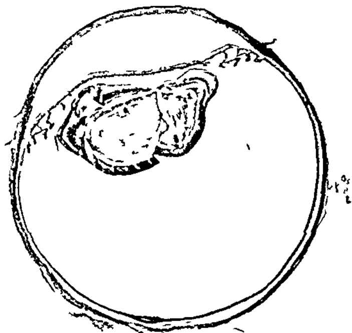


Fig 4
Section of an eye treated intravitreally with 5 0 mg of carbenicillin 10 hours after inoculation with *Pseudomonas aeruginosa* Four weeks following treatment the retina remains intact and the vitreous is free of inflammatory infiltrate (Hematoxylin eosin × 9)

iritis and corneal edema opacification of the vitreous prevented fundus examination. The infection progressed to phthisis bulbi in 2 to 3 weeks yielding cultures positive for Pseudomonas up to 6 to 8 weeks While the vitreous routinely became opacified the anterior chambers tended to remain much more clear for the first 72 hours allowing the pus filled vitreous to be seen

Eyes treated with intravitreal carbenicillin at 3 hours were all quiet by 2 to 3 days the only evidence of infection being opacification in the vitreous which showed good vitreal organization with diaphanous membranes at approximately 1 week One of these eyes showed speckling on the posterior lens which did not subsequently clear Two of the 3 hour eyes were fixed for histologic section and showed no microscopic changes None gave positive cultures when sacrificed The eyes treated at 6 hours after inoculation showed more organization of the

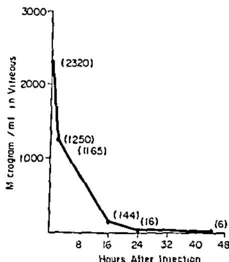


Fig 3
Clearance from the vitreous of a 3 0 mg dose of carbenicillin injected intravitreally into a noninflamed rabbit eye

apparent (Fig 2) One 5 0 mg eye was subjected to electroretinography and applanation tonometry both before and after carbenicillin injection and was found to have a normal ERG and a normal intraocular pressure

Clearance

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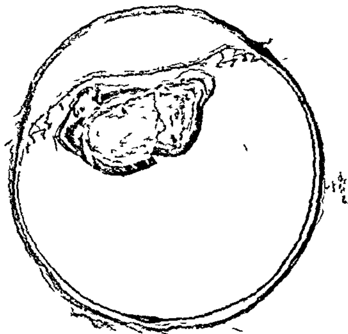


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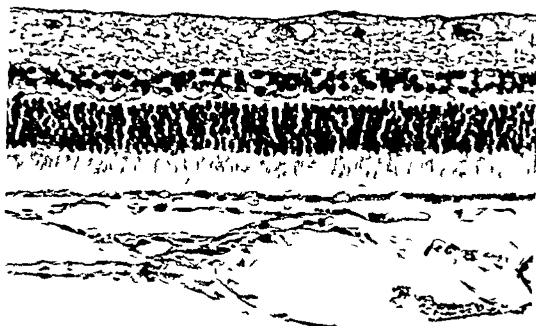


Fig 5
Histologic section at 4 weeks of an infected eye treated with 5 0 mg of carbenicillin intravitreally 10 hours after inoculation Note normal retina (Hematoxylin eosin × 00)

vitreous with larger vitreal abcesses which were stable by 48 hours Cataracts formed in two of the six eyes Four of the 6 hour eyes were fixed for histopathologic study and three showed no ocular tissue changes One showed minimal retinal degenerative changes The eyes treated at 10 hours after inoculation generally revealed the same changes as the 6 hour group but with more iritis more cataract formation and greater vitreal organization at 48 hours Subsequently over the next 3 weeks four of these eyes stabilized (Fig 4) while the other two were cultured to retrieve Pseudomonas and had progressed to phthisis Histologic sections of the noninflamed eyes showed normal retinal structure (Fig 5) Thus one third of the eyes treated at 10 hours after inoculation did not recover whereas treatment halted the infection in the other four Of the six eyes treated at 24 hours two eyes recovered appearing normal clinically histologic study of these revealed normal retinas The remaining eyes in the 24 hour group the 36 hour group and the control group all progressed to phthisis both clinically and histologically (Fig 6)

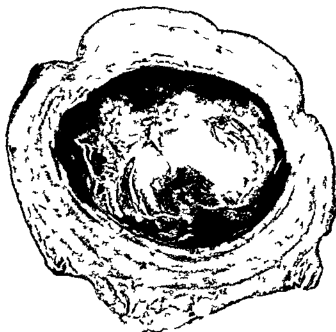


Fig 6
Gross appearance at 4 weeks of a control eye showed massive purulent exudate within the vitreous and destruction of the ocular structures (Hematoxylin eosin ×9)

Discussion

Intravitreal injection of a small dose of carbenicillin is a safe procedure and is uncomplicated by the toxic changes that may involve the retina or lens when higher doses are used Interestingly when using carbenicillin in the 8 to 10 mg range minimal retinal toxic changes were seen histologically in certain of the specimens while other specimens from this same dose range were normal by the same criteria This could be explained on the basis of variations in local flow and current characteristics A 5 mg dose was used for treatment of the intra vitreal infection to insure bactericidal levels in the vitreous for 24 hours and also because this dose remains well below a dosage level associated with ocular toxic reactions

Rabbit eyes inoculated with 500 to 1 000 Pseudomonas organisms showed *definite signs* of infection within 6 hours and had developed a severe endophthalmitis within 10 hours Since Pseudomonas produces a rapidly *progressing* endophthalmitis and since there is an inverse relationship between the onset of therapy and the severity of the ensuing infection and sequelae it is not surprising that there was such a marked difference clinically and histologically in the degree of vitreal abcess and organization depending on the time lapse between infection and treatment While the abcesses that appear following treatment at the first signs of infection are small and will eventually disappear the abcesses that occur when treatment is delayed until 10 hours are very large occupying a large portion of the vitreous do not organize well and in this study one third of the eyes in this group progressed to phthisis bulbi The successful treatment then of this experimentally induced infection is dependant upon intravitreal injection of carbenicillin at the first sign of vitreal clouding

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Aus der Augenklinik des Bereiches Medi in der Universitat Rostock (Direktor OMR Prof Dr sc med G Pietruschka) und der Hals Nasen Ohren Klinik des Bereiches Medi in der Universitat Rostock (Direktor Prof Dr sc med K Dietzel)

Herrn OMR Prof Dr sc med Pietruschka zum 60 Geburtstag gewidmet

BEITRAG ZUR FRAGE DER FUNKTIONELLEN UND REGULATORISCHEN BEEINFLUSSUNG DES AUGES BEIM MORBUS MENIÈRE

VON

H P VICK U VICK und U HEINE

Bei 24 in der Univ HNO Klinik Rostock stationar behandelten Patienten mit einem Morbus Meniere werden ophthalmologische Befunde ein schließlich funktionsdiagnostischer Daten erhoben und analysiert Be sonderer Wert wurde auf die Aussage gelegt daß keine funktionellen oder organischen Augenbefundsanderungen zwischen den Untersuchungen im Meniere Anfall und im beschwerdefreien Intervall bestanden

Key words morbus Meniere eye – and mb Meniere glaucoma

Immer wieder wird in der Literatur auf den Zusammenhang von Labyrinth hydrops und Augenfunktionsstorungen hingewiesen Jedoch gehen die Meinun gen zu diesem Problem von absoluter Bejahung uber zuruckhaltende Auf fassungen bis zur Ablehnung eines Zusammenhanges gemeinsamer Funktions störungen weit auseinander

1861 beschrieb Prosper Meniere eine anfallartig verlaufende Symptomentrias die durch Drehschwindel dauerndes oder intermittierend anschwellendes Ohrensausen und fluktuierende Horstorungen mit fortschreitendem Horverfall charakterisiert ist (Burian 1973) Der Otologe Knapp (1871) vermutete als

erster eine intralabyrinthare Drucksteigerung als Ursache des Meniereschen Anfalls und der Physiologe Hensen (1880) verglich den Labyrinthhydrops zuerst mit der intraokularen Druckerhöhung Seit von Hensen (1880) und später von Rampoldi (1889) auf die Beziehungen zwischen Menierescher Erkrankung Schwerhörigkeit und Glaukom hingewiesen wurde haben sich zahlreiche Untersucher mit dem Vergleich der Druckverhältnisse in Auge und Innenohr und einer gemeinsamen Ätiopathogenese von Dysregulationen befaßt Daß es bis heute noch nicht zu einer einheitlichen Beantwortung dieser Fragen gekommen ist deutet auf die Kompliziertheit dieses Problems hin. Relativ häufig ist in Kasuistiken das synchrone Auftreten von akuten intraokularen Drucksteigerungen bei der Meniereschen Erkrankung beschrieben Umgekehrt soll es beim akuten *Glaukom zu Meniere ähnlichen Symptomen* kommen so daß dafür sogar die Bezeichnung extraokulares Glaukomsymptom (Tille) geprägt wurde

Die anatomischen Analogien von Auge und Innenohr insbesondere das Vorhandensein eines Flüssigkeitskörpers in beiden Sinnesorganen lassen an gemeinsame ätiologische *Momente bei der hydrodynamischen Dystonie* denken als die in grober Verallgemeinerung der komplizierten Regulationsmechanismen sowohl das Glaukom wie auch die Meniere sche Erkrankung aufgefaßt werden (Sokolowski (1953) Godtfredsen (1950) Mc Grath Magdalena Castineira (1956) Ten Doesschate (1955) Breuninger Lampe et al (1963) & Massimeo et al (1958))

Als mögliche gemeinsame Ursache für Druckdysregulationen im Labyrinthsystem bzw im Auge werden von Ciurlo gefäßneurotische Störungen vermutlich bedingt durch Schädigung des Sympathikus angenommen Beretta (1957) sieht einen Zusammenhang durch neurovegetative Störungen der Kapillardurchlässigkeit sowohl im Innenohr als auch *in der Uveamembran* Ähnliche gemeinsame auslösende Faktoren werden auch dem von zahlreichen Autoren beschriebenen Zusammenhang zwischen Glaukom und Perzeptionsschwerhörigkeit zu Grunde gelegt. Auch hierbei sind die Meinungen bisher stark *differierend Zu Fragen dieses Zusammenhanges* möchten wir auf die Arbeiten von Pietruschka (1959) sowie Vick und Vick (1970) verweisen

Untersuchungsmethode

Diese Betrachtung der Pathogenese des Morbus Meniere und die mannigfaltigen Auffassungen verschiedener Autoren veranlaßten uns die in der Universitäts HNO Klinik Rostock stationär behandelten an Morbus Meniere erkrank

ten Patienten ophthalmologisch zu untersuchen um an einem möglichst großen allseitig fundiert diagnostizierten Patientengut Aussagen über Augenbefunde insbesondere das Augendruckverhalten bei Patienten mit Morbus Menière treffen zu können Soweit die Möglichkeit bestand erfolgte sofort nach Aufnahme von akut erkrankten Patienten in der Universitäts HNO Klinik eine ophthalmologische Untersuchung die jedoch je nach Heftigkeit des Menière Anfalls variiert werden mußte So konnten z B bei Patienten mit ausgeprägten vegetativen Beschwerden wie Schweißausbruchen, Übelkeit Erbrechen bzw starken Tinnitus oft nicht sofort ophthalmologischfunktions diagnostische Untersuchungen durchgeführt werden Eine sofortige Messung des intraokularen Druckes durch Impressionstonometrie erfolgte jedoch ausnahmslos im Menière Anfall In der Regel wurden alle Patienten zweimal ophthalmologisch untersucht zunächst während der akuten Anfallsphase mit variablem Untersuchungsprogramm und dann ein zweites Mal im beschwerdefreien Intervall Diese Doppeluntersuchung sollte mögliche Befunddifferenzen aufdecken

Die augenärztliche Untersuchung bestand neben der Erhebung einer speziellen Anamnese und einem allgemeinen Augenbefund insbesondere in der Gewinnung möglichst vieler und genauer funktioneller Daten wie peripheres und zentrales Gesichtsfeld Farbensinn und Adaptation Besonderes Interesse galt auch dem Forschen nach einem latenten oder manifesten Glaukom Hierzu wurden neben Tensionsmessungen mittels Impressions und Applanationstonometrie auch alle Patienten gonioskopiert und diaphanoskopiert sowie die Bulbusrigidität mittels Differentialtonometrie bestimmt In begründeten Fällen wurden Glaukomprovokationsproben durchgeführt

Untersuchungsergebnisse

In den Jahren 1970–73 konnten 24 Patienten die an Morbus Menière erkrankt waren und zum großen Teil schon mehrere Jahre in HNO ärztlicher Behandlung stehen augenärztlich untersucht werden Es handelt sich um 10 Männer und 14 Frauen im Alter von 30 bis 64 Jahren Das Durchschnittsalter betrug 42 Jahre

Die audiologische Untersuchung ergab 17mal eine unilaterale und in 7 Fällen eine beidseitige Innenohr bzw kombinierte Schwerhörigkeit In der überwiegenden Mehrzahl fanden wir im Audiogramm den pancochlearen Kurventyp in 7 Fällen mit der typischen Wannenform

Die Vestibularisprüfung erbrachte folgende Befunde In 10 Fällen wurde

ein Tonusuberwiegen des kranken und 11mal des gesunden Ohres festgestellt Nur bei einem Patienten zeigte sich ein seitengleich erregbares Labyrinthorgan und in 2 Fallen wechselten Nystagmusform und Schlagrichtung so mannigfaltig daß eine Auswertung nicht moglich war

Der Blutdruck war bei 17 Patienten normoton bei 3 hyperton und in 4 Fallen hypoton

Erwahnt werden muß auch noch daß von den 24 untersuchten Patienten in 18 Fallen rontgenologisch pathologische Halswirbelsaulenveranderungen nachgewiesen wurden

Die ophthalmologische Untersuchung ergab bei 23 der 24 untersuchten Patienten normale intraokulare Druckwerte nicht uber 21 mm Hg Auch der Vergleich der Tensionswerte die wahrend des Anfalls und in dem beschwerdefreien Intervall bestimmt wurden erbrachte keine Differenzen die uber eine physiologische Schwankungsbreite von 2–3 mm Hg hinausgingen

Eine Seitendifferenz im Verhalten des intraokularen Druckes entsprechend der Seite des Cortischen Organs von welchem der Meniere Anfall ausgeht fand sich nicht

Wir registrierten auch regelrechte Werte bei der Bestimmung der Bulbusrigiditat und keine auffalligen Gonioskopie und Irisdiaphanoskopiebefunde

Bei einem 39 jahrigen Patienten mit spontanen Druckwerten bis 24 mm Hg und teilweise positiven Glaukomprovokationstests bei erhaltener Funktion und zentral exkavierten Papillen wurde ein Praeglaukom bds diagnostiziert Bei diesem Patienten ist der Morbus Meniere mit einer mittelgradigen Innenohrschwerhorigkeit vom pancochlearen Typ seit 3–4 Jahren bekannt Es ist von Interesse daß auch bei diesem Patienten der zu einer Dysregulation des intraokularen Druckes neigt, wahrend des Meniere Anfalls keine auffalligen Druckschwankungen auftraten

Soweit durchfuhrbar und auf Grund des Allgemeinzustandes zumutbar wurden bei den Patienten Gesichtsfeld und kampimetrische Untersuchungen auch mit farbigen Testmarken durchgefuhrt In keinem Falle konnten periphere Gesichtsfeldausfalle oder Skotome nachgewiesen werden

Die Untersuchung des Farbensinns mit den Farbtafeln nach Velhagen Ishihara sowie mit dem Anomaloskop nach Nagel ergab bei zwei mannlichen Patienten eine Storung im Rot Grun Bereich im Sinne einer Deuteranopie bzw Deuteranomalie Beziehungen zwischen diesen angeborenen Farbsinnstorungen mit typischen Einstellungen am Anomaloskop und der vestibularen Storung bestehen wohl nicht Adaptationsuntersuchungen mit dem Zeiss Nyktometer und dem Registrieradaptometer nach Prof Hartinger konnten bei allen Patienten nur im beschwerdefreien Intervall durchgefuhrt werden und ergaben keine pathologischen Befunde

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Zusammenfassung und Diskussion

Um dem in der Literatur immer wieder zitierten ätiologischen Zusammenhang zwischen dem labyrinthären Hydrops und pathologischen funktionellen wie auch organischen Augenbefunden nachzugehen, wurden 24 Patienten, die an einem Morbus Menière leiden, eingehend augenärztlich untersucht. Wert legten wir auf die Aussage, daß keine funktionellen oder organischen Augenbefundsänderungen zwischen den Untersuchungen im Menière-Anfall und im beschwerdefreien Zeitraum bestanden. Besondere Aufmerksamkeit wurde dabei dem Verhalten des intraokularen Druckes geschenkt. Bei fünf als Glaukomprovokationstest durchgeführten Flüssigkeitsbelastungsproben wurde in keinem Falle ein Menière-Anfall bei den disponierten Patienten provoziert.

Wir konnten die von Bietti und Porta (1952) beobachtete Parallelität von Augendruckzunahme und vestibulärer Reaktion bei Flüssigkeitsbelastungsproben nicht nachweisen.

Auch die übrigen funktionellen Leistungen des Sehorgans wiesen während oder nach der hydropischen Reizung des Cortischen Organs keine Beeinträchtigungen auf, soweit durch die mehr oder weniger ausgeprägte vegetative Allgegemeinreaktion eine verwertbare ophthalmologische Befunderhebung möglich war.

Wir möchten deshalb auf Grund unserer Untersuchungsergebnisse größte kritische Zurückhaltung bei der Beurteilung eines Zusammenhanges zwischen dem Morbus Menière und pathologischen Augenveränderungen, insbesondere auch in bezug auf die gleichzeitige intraokulare Druckdysregulation üben. Die Folgen des erhöhten Druckes im Auge bzw. Ductus cochlearis sind durchaus vergleichbar und führen in Abhängigkeit von Druckhöhe und Dauer durch hypoxaemische Schädigung der nervalen Elemente zu irreversiblen Schädigungen in beiden Organen. Ein gemeinsamer Faktor jedoch, der in beiden Organen signifikant häufig ätiologisch auslösend wirkt, kann von uns bisher nicht anerkannt werden.

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Methods

Two hundred and twenty cataract extraction procedures were performed under local anesthesia and with identical surgical technique Acetazolamide and osmotic therapy (glycerin by mouth) were given pre operatively to achieve a lower intraocular pressure Pressure was applied in *all* cases to the globe for about 1 min following retrobular anesthesia to ensure a more thorough diffusion of anesthetic solution within the muscular cone

Local anesthesia and akinesia were administered using 2 % lignocaine hydro chloride with the addition of 6–8 drops of adrenaline 1 1000 and 300 units of hyalase for the retrobulbar injection

The operating table was tilted in such a way as to attain an anti Trendelen burg plane The angle varied between 6°–8 even higher for big and heavy patients but never more than 10

Results

There was no vitreous loss in any of the patients operated upon in this series A marked concavity of the anterior face of the vitreous as well as a pontaneous filling of the anterior chamber with air as the lens was delivered were often observed Vitreous bulging was seen in 2 7 % of the patients but in none was vitreous prolapse noted

Discussion

Loss of vitreous is an important complication in cataract extraction leading both to immediate and delayed consequences The presence of vitreous strands at the wound edges prevents good apposition and causes faulty healing of the corneoscleral incision The sequelae of these events are difficult to treat fibrotic overgrowth in the post operative period with subsequent distorted or drawn up pupil or apposition of the vitreous face to the endothelium of the cornea follow ed by bullous keratopathy Eyes with vitreous loss are *more prone to develop* secondary glaucoma and retinal detachment Cystoid macular edema frequently follows vitreous loss and may persist indefinitely

Although a great deal of attention has been paid to the management of the wound by vitreal aspiration and excision at the time of vitreous loss *prevention* of this complication is still the main object The rationale of inducing ocular

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PREVENTION OF VITREOUS LOSS IN CATARACT EXTRACTION

BY

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Cataract extraction was performed in the supine head up (anti Trendelenburg) posture in 220 patients without vitreous loss The rationale for this surgical posture approach as a useful additional technique in preventing vitreous loss is discussed Other clinically accepted methods for the prevention of this complication in cataract surgery are summarized

Key words surgery – cataract – head up posture

Special attention has always been paid to the prevention of vitreous loss in cataract surgery and a large number of papers have been devoted to this subject The ocular hypotony induced during adult cataract extraction is believed to have a beneficial effect in preventing this complication (Gartner 1959 Townes 1965 Jaffe 1968) Total akinesia to preclude extraocular muscle contraction (Kornblueth Jampolsky Tambler & Marg 1960) as well as the position of the patient during surgery (Bustillo 1971) are other pre operative measures for preventing loss of vitreous

The present report describes a series of consecutive cataract extractions performed in the supine head up (anti Trendelenburg) posture without vitreous loss Suggestions are made regarding the underlying mechanism that prevents vitreous loss when this postural variation is used in cataract surgery

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but none in 107 extractions when the patient s head was maintained at maximum elevation and epinephrine injected subconjunctivally He postulated that optimum head elevation and dosage of subconjunctivally administered epinephrine minimize ocular and orbital vascular congestion in cataract surgery In an ophthalmoscopic study of the fundus immediately after lens delivery and before restoration of the intraocular pressure Kirsch & Singer (1973) revealed infoldings of the sclera choroid and retina in 84% of the eyes examined Such infoldings were either radial (with regard to the optic disc) circumferential or both These infoldings could be made to flatten or disappear by maneuvers which increase the intraocular pressure

The orbit is drained by three principal routes (1) backwards through the superior and inferior ophthalmic veins to the cavernous sinus (2) forwards through the anastomosis of ophthalmic veins with the angular vein into the facial system (3) downwards through the inferior orbital tissue to the pterygoid plexus The anti Trendelenburg posture is accompanied by a freedom from pressure on the jugular veins and probably by an increased orbital venous drainage Although not specifically investigated in this clinical study it is suggested that an increased orbital depletion with a sequential lessening of the retrobulbar tissue pressure is the effect of this supine head up position The rationale for this postural approach was based on a strong clinical impression that vitreous will not run upward until something pushes it Accordingly an excessive increase of choroidal volume or a substantial pressure exerted toward an open scleral shell by congested orbital content will facilitate subsequent vitreous expelling The results of this report suggest the beneficial effect of the anti Trendelenburg posture as a useful additional measure in the prevention of vitreous loss during cataract extraction In order to substantiate this limited experience larger controlled studies and measurement of retrobulbar tissue pressure and its secondary effects on the configuration of the surgicaly open eye are necessary

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hypotony by either acetazolamide osmotic therapy or ocular massage before cataract extraction to reduce the incidence of vitreous loss has largely been described (Kirsch & Steinman 1955 Gartner 1959 Friedman Byron & Turtz 1962 Hill 1964 Jaffe & Light 1965 Townes 1965 Jaffe 1968 Kornblueth & Gombos 1962, Seeger & Lewis 1964 Robbins & Galin 1969) However in a recent study Galin Robbins & Obstbaum (1971) inferred that hypotony *per se is not of benefit in reducing the possibility of vitreous loss but that it is of value* when induced by hyperosmolar therapy which reduces vitreous mass The investigators performed limbal incisions in rabbits eyes to mirror the initial steps of a lens extraction They found that the vitreous body loses water and decreases in weight simply by opening the anterior chamber Previous digital massage did not increase this vitreous weight change but hyperosmolar therapy did The validity of carbon anhydrase inhibitor drugs administered pre operatively is less feasible for testing Since their role is limited to the suppression of aqueous secretion these drugs cannot provide a traceable effect in preventing vitreous loss However *Urrets Zavalia (1962) described a particularly beneficial* effect by using Diamox® in cataract surgery This drug seems to alter the *colloidal state of the vitreous removing part of its water content thus increasing* its viscosity

Although a soft eye during cataract surgery is an important factor in *reducing* the incidence of vitreous loss and is the subject of much research prevention of external pressure on the globe is equally important in avoiding this complication As soon as the anterior chamber is open the intraocular pressure falls to zero and any minor extraocular positive pressure exerted against the globe will prolapse the vitreous body in a forward direction

It becomes apparent why adequate steps are required to prevent scleral infolding in a surgically opened eye Consistent with this deep akinesia – to preclude extraocular muscle activity and the resulting shortening of the scleral shell – is an important measure for avoiding vitreous loss (Kornblueth Jampolsky Tambler & Marg 1960)

In the present series the surgical postural anti *Trendelenburg variation* was chosen for cataract extraction as an additional technique against vitreous loss Whether such a procedure is of any significance in precluding *posterior segment* infolding by elevated retrobulbar tissue pressure has not yet been established although there is some evidence which suggests this to be so Birch *Hirschfeld* (1930) found that the antero posterior position of the eye changes under certain circumstances in the normal person His work is of particular interest since it shows the importance of orbital venous drainage in regard to the position of the eye in the orbit In a recent study Bustillo (1971) encountered six instances of vitreous loss in 78 cataract extractions with the patients in the horizontal position

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STIMULUS DURATION AND THE OSCILLATORY POTENTIALS OF THE HUMAN ELECTRORETINOGRAM

BY

LILLEMOR WACHTMEISTER

The effect of duration of stimulus light on the threshold of oscillatory and the slow (a and b wave) potentials of the human ERG were assessed on light adaptation to light stimuli.
On weak light adaptation induced by stimuli given at 2 min intervals there was a temporal summation of the oscillatory potentials over a range of at least 2 log units (4 msec 400msec). The threshold of the a wave and b wave was dependent on duration up to a critical duration of about 40 msec.
On strong light adaptation by repetitive light stimulation with 15 sec intervals the oscillations were determined by intensity alone over a range of [illegible] log units. The a wave revealed a similar behaviour whereas the b wave integrated stimuli in the temporal domain up to about 10 msec.
Of main importance the results provide evidence which is suggestive of temporal integration and discrimination being independently governed by *the neurons (probably at the inner plexiform layer)* which generate the oscillatory potentials.

Key words electroretinography – oscillatory potentials – light adaptation.

Psychophysical data show that in the dark adapted eye there is a critical duration of light stimulus below which the relationship $I \times t = c$, the Bunsen Roscoe law holds if I *is the stimulus intensity and t* the duration of light stimulus (Barlow 1957). In light adaptation this critical duration decreases i. e. the temporal summation is less (Commichau 1955 Barlow 1958).

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two stimuli in each series induced a comparatively strong state of light adaptation at the point of time when the third stimulus light was delivered The intensity of all three lights increased logarithmically in steps of 0.3–0 4 log units The procedure was repeated with duration of light stimulus varying over a range of 2 log units

II In another series of experiments the procedure was very similar to the procedure described above light stimuli were given with longer intervals i e 2 min The retinal state of light adaptation caused by the previous light stimuli was then comparatively weak when the next stimulus appeared

Results

The threshold of the ERG was recorded in response to light stimulus of different durations varying over 2 log units Three consecutive light stimuli were given with short (15 sec) or long (2 min) intervals and the ERG recorded in response to the third light stimulus The intensity of all three light stimuli varied from log I = –4 0 to $\log I_1 = 0$ All ERGs shown in the pictures were recorded from the right eye of the same subject

I *Strong light adaptation caused by stimulus light (short intervals)* With the shortest duration of stimulus light (4 msec) a b wave of 42 μV appeared in response to stimulus intensity of log I = –4 0 (Fig 1) Using stimulus of longest duration (400 msec) a b wave of 72 μV was recorded in response to stimulus light of log I = –4.3 An a wave of 22 μV was recordable in response to log I = –2 3 when stimulus of shortest duration was used With stimulus of 400 msec duration an a wave of 56 μV was recorded in response to stimulus light of log I = –2 3

The oscillatory potentials were elicited when stimulus intensity of log I = –2 3 and shortest duration (4 msec) as well as longest duration (400 msec) were used The most prominent oscillations were demonstrated with stimulus of maximal intensity and a duration of 4 and 40 msec

Consequently the threshold of the a wave and the oscillatory potentials did not show any significant change when light stimulus was changed over a range of 3 log units (Fig 2) There was a tendency of temporal summation of the b wave up to 10 msec duration Using longer stimulus duration the threshold only seemed to be determined by stimulus intensity (Fig 2)

II *Weak light adaptation caused by stimulus light (long intervals)* Using shortest duration of light stimulus (4 msec) a b wave of 76 μV appeared in response

A similar critical duration also characterizes the conditions for eliciting electrical responses of invertebrate and vertebrate eyes (Adrian & Matthews 1927 Hartline 1928, 1934) as well as of human eyes (the a and b wave) (Johnson & Bartlett 1955 Alpern & Farris 1956 Wirth 1956 van Lith 1966)

Little is known of the relationship of the oscillatory potentials of the human electroretinogram to the duration of light The present investigation was undertaken to make evident the dependence of the oscillatory potentials of the human electroretinogram on the duration and intensity at different states of light adaptation

Apparatus and Methods

The apparatus and general procedure in this study are similar to those previously described by Wachtmeister (1974) The maximum luminance of the photostimulator (XBO 900 W/2 Osram Oriel xenon dc arc lamp) was now set to approximately 3 x 10 photopic cd/m The colour temperature of the stimulus light corresponded to about 6 000°K

A pulse generator (Scandia Metric Exact Model 126) regulated an electromagnetic shutter which regulated the duration of light stimulus The duration varied from about 4 to about 400 msec

A Lawwill Burian contact lens (Lawwill & Burian 1966) was used as an active and reference electrode respectively The electric signal was amplified using the same electronic equipment calibration and band pass of recording system as previously described (Algvere Wachtmeister & Westbeck 1972) The electric response was displayed on a double beam cathode ray oscilloscope (Hewlett Packard 132 A) Photographic records provided the data The methods of measuring the amplitudes were previously described by Algvere et al 1972

Procedures

Experiments were done with three young healthy subjects (two women and one man) Central visual acuity visual fields visual sensitivity and colour vision were normal The pupil was dilated with Mydriacyl® (Alcon lab) to more than 6 mm in diameter Topical anaesthesia was established by Novesin® (Wander) The fellow eye was occluded

After at least 30 min of dark adaptation the following two procedures were performed

I A series of three light stimuli of the same intensity was given at intervals of 15 sec The ERG in response to the third stimulus light was studied The first

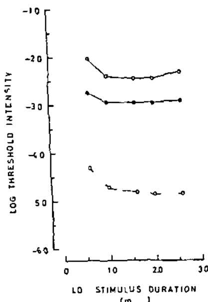


Fig °

Electric threshold of the oscillatory potentials the a and b wave in relation to duration of light stimulus The procedure was the same as in Fig 1 Threshold criterion was 33 μV for the a and b wave and 2 5 a u (arbitrary units) for the oscillatory potentials Average of three subjects Black circles oscillatory potentials Open circles continuous line a wave Open circles – dashed line b wave

to light stimulus of log I =–4 3 (Fig 3) A b wave of 34 μV was induced by light stimulus of log I =–5 3 when stimulus duration was 40 msec (or longer) In response to light stimulus of log I =–? 3 and 4 msec duration an a wave of 44 μV appeared When the duration increased to 40 msec (or longer) an a wave of 34 μV was recorded in response to log I =–3 3

The oscillatory potentials were elicited by stimulus light of log I =–2 0 and 4 msec duration When the longest duration (400 msec) was used distinct oscillations appeared in response to light stimulus of log I_s =–3.3 The oscillations were most easily elicited when stimulus of maximal intensity and duration of 40 msec were used

Evidently the threshold of the slow (a and b wave) as well as the oscillatory potentials changed as the stimulus duration increased (Fig 4) The slow potent

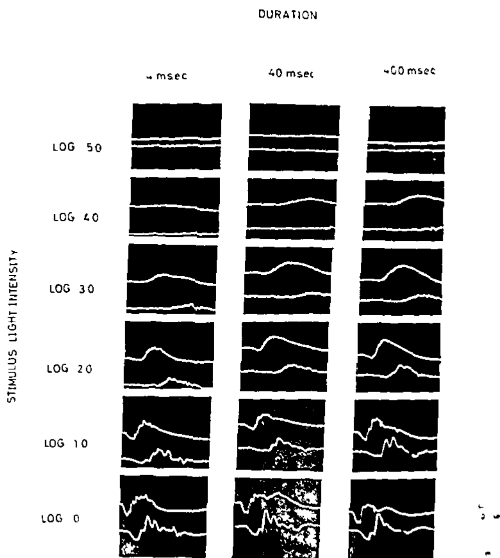


Fig 1

Threshold and relation to stimulus light intensity of the amplitude of the slow (a and b wave) and the oscillatory potentials for three different durations of stimulus The duration was varied over a range of 2 log units A series of three light stimuli of the same intensity was given The intensity of all three light stimuli increased in a logarithmic scale There was 15 sec between each stimuli although the intensity of the stimulus varied Each ERG was displayed in a slow cathode ray sweep speed and low amplification (0 2 mV/cm 20 msec/cm) (upper trace) and simultaneously in a rapid cathode ray sweep and high amplification (0 1 mV cm 10 msec/cm) (lower trace) The ERG in response to the third stimulus light in a series of three is illustrated in each picture

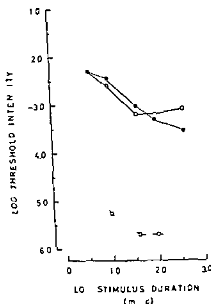


Fig 4

Electric threshold of the oscillatory potentials the a and b wave in relation to duration of light stimulus The procedure was the same as in Fig 3 The same x and y axis criterion amplitude and symbols as in Fig 2 Average of three subjects

III *Comparison between the effect of stimulus duration in strong and weak light adaptation (induced by light stimuli)* Fig 5 discloses the comparative effect of different light adaptation induced by stimuli delivered at short or long intervals The range of total temporal summation of the b wave is somewhat less in strong than in weak light adaptation induced by stimulus lights In strong light adaptation induced by light stimuli there was a less abrupt intersection of the horizontal and sloping straight lines

The threshold of the oscillatory potentials was determined by intensity of stimulus light alone when short intervals between stimuli were used When longer intervals and weak light adaptation by stimuli were used the threshold was dependent on intensity as well as duration of stimulus i e temporal summation existed over at least ? log units

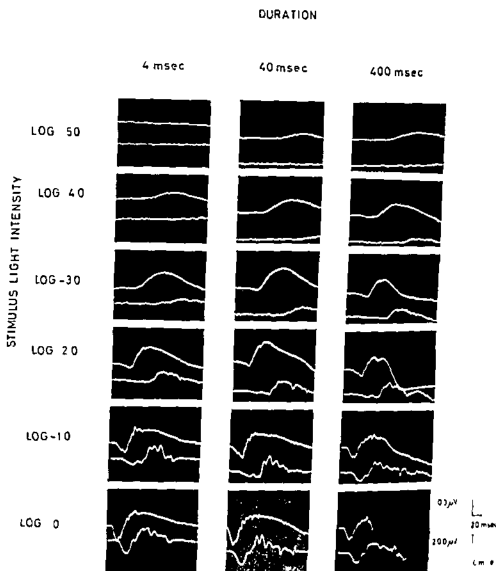


Fig 3

Threshold and relation to stimulus light intensity of the amplitude of the slow (a and b wave) and the oscillatory potentials for three different durations of stimulus Weak light adaptation caused by stimulus light which was given at 2 min intervals In other respects the procedure was similar to that described in Fig 1

ials revealed a temporal summation up to 40 msec duration whereas the oscilla tory potentials integrated temporally up to the longest duration (400 msec) used (Fig 4) The decrease of the log threshold intensity was approximately linear the slope of function being about 0 6

recent anatomical and physiological evidence of different origins of the a and b waves from the oscillatory potentials (Werblin 1969 Miller & Dowling 1970 Ogden 1973 see Wachtmeister 1972 for further references)

On adaptation to background light the slope of function of incremental thresholds of the oscillations was shallower than that of the b wave which was interpreted as a much less extensive temporal summation of the oscillatory potentials than that of the b wave (Wachtmeister 1973) The present study also provides evidence which suggests that there is less temporal integration of the oscillations than of the b wave in strong light adaptation induced by stimuli

During the used conditions of weak light adaptation induced by stimuli the spectral curve of retina approximated the scotopic one (although photopic activities cannot be excluded) as has been previously shown (Wachtmeister 1974) The sensitivity of the oscillations increased with duration By increase of stimulus duration lateral inhibitory interactions seemed to be strengthened (Nachmias 1967 1968 etc) On the basis of microelectrode depth studies of the primate (monkey) retina Ogden (1973) suggested that the oscillatory potentials are generated by membranes of the inner plexiform layers involving the axon terminals of the bipolar cells the processes of the amacrine cells and the dendrites of the ganglion cells Korol Meyer & Leuenberger (1974) recently demonstrated a parallel lesion of the amacrine cells (autoradiographically) and the loss of the oscillatory potentials of the ERG by injections of glycine into the vitreous body of the rabbit eye By experiments with simultaneous contrast Rockefeller et al 1973 found that an inducer flash raised the psychophysical threshold as a function of its duration as well as its separation from the test Thus the effect of duration may be interpreted as a greater temporal convergence and increase of neural out put by the neurons of the inner plexiform layer

When light adaptation increased by repetetive stimulation with shorter intervals the sensitivity of the oscillations depended entirely on stimulation intensity i e temporal summation was ceased The retinal state of adaptation had changed to a predominately photopic one which has recently been demonstrated (Wachtmeister 1974) The spectral sensitivity of the responses is then largely governed by cones By comparison with the rods the cone receptor system is specialized for resolution of stimuli in time as demonstrated by higher CFF of cones This has been shown both psychophysically and electroretinographically (Babel & Monnier 1949 Dodt & Wadensten 1954) The cone system also shows less convergence of receptor – bipolar ganglion cells (Polyak 1941 Kolb 1970) in comparison with rods Thus there is probably a difference in spatial organization of the separate types of neurons generating the a wave b wave and the oscillatory potentials in photopic and scotopic conditions Thus the lack of

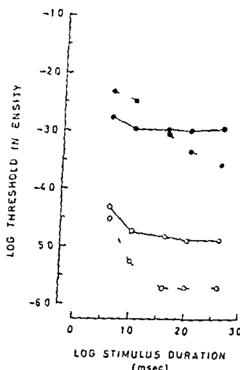


Fig 5

Comparison between the electric threshold curve of the b wave and the oscillatory potentials for three different durations of stimulus as shown in Figs 2 and 4 Black circles oscillatory potentials Open circles b wave Continuous line strong light adaptation by stimuli given at an interval of 15 sec Dashed line weak light adaptation by stimuli given at an interval of 2 min

Discussion

It appears from Fig 5 that a change in the retinal adaptation affected the temporal characteristics of the b wave The range of temporal summation showed a decrease as the light adaptation increased This result parallels that of previous studies concerned with duration effects of the b wave although in that material the temporal characteristics were studied during adaptation to background light of different intensities (Alpern & Faris 1956 van Lith 1966) The oscillations on the other hand showed no sign of temporal summation in strong light adaptation whereas the change of sensitivity (reciprocity of threshold) with duration increased in weak light adaptaton Thus the rapid oscillatory potentials behaved entirely differently to that of the slow potentials (a and b wave) as stimulus duration varied These observations are in accordance with

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temporal integration during strong light adaptation by stimuli seems to support the hypothesis that the oscillations reflect lateral interactions probably the result of activity in a feed back pathway at the inner plexiform layer

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Methods

Central corneal thickness was measured with the Haag Streit pachometer as previously reported (Ehlers & Kruse Hansen 1971) The values given are the averages of five readings Intraocular pressure was measured with the Goldmann applanation tonometer and the Schiøtz indentation tonometer The glaucoma diagnosis was based on the visual field defects excavation of the optic disc and on a pressure reducing effect of anti glaucoma treatment Tonography has not been used as the diagnosis could well be established without this – and as the foundations for the calculations may be unreliable in these eyes Differences between groups were tested statistically by the t test. The frequency distribution of corneal thickness does not differ from the normal Gaussian curve (Kruse Hansen 1971)

Case histories

1 (AKH 800696) 76 year old man with visual field defects recognized for 2 years Treated with pilocarpine in spite of normal intraocular tension at all examinations Visual acuity OD 0 8 + 1 00 90° OS 0 6 + 2 00 65° Biomicroscopy was normal with open chamber angles Ophthalmoscopy showed glaucomatous cupping of the discs Perimetry revealed arcuate defects (Fig 1) Without treatment the intraocular tension was OD 16 mmHg OS 14 mmHg by applanation tonometry It was measured 3 times daily for 7 days and was never above 20 mmHg A waterloading test showed no significant increase in pressure Indentation tonometry OD 5 5/5 5 g 10 0/10 0 g OS 5 0 5 5 g 10 0/10 0 g Central corneal thickness OD 0 452 mm OS 0 452 mm Neurological examination and plain radiography of the skull with laminography of the sellar region showed nothing abnormal EEG was normal Treatment with pilocarpine was continued

(AKH 2 0315) 58 year old woman complaining of headache Examination showed VOD 0 6 1 00 sph VOS 0 67–0 50 sph Biomicroscopy including gonioscopy was normal ophthalmoscopy showed glaucomatous cupping of the discs Perimetry revealed arcuate defects (Fig 1) Applanation tonometry OD 14 mmHg OS 15 mmHg A waterloading test was negative Central corneal thickness OD 0 473 OS 0 478 mm Neurological examination and plain radiography of the skull showed nothing abnormal EEG was normal

3 (AKH 0 0501) 7 year old man complaining of decreasing vision during one year Examination showed VOD hand movements in front of the eye VOS 0 5 emmetropia Biomicroscopy including gonioscopy was normal ophthalmoscopy showed glaucomatous cupping and degenerative changes in the macular regions Perimetry showed severe field defects (Fig 1) suggestive of late glaucoma Applanation tonometry OD 17 OS 15 mmHg Schiøtz tonometry both eyes 4 0 5.5 g 9 0/10 0 g 13 0/15 g The applanation pressure measured several times daily during 6 days was never above 19 mmHg Waterloading test showed no increase in pressure but an epithelial oedema developed Central corneal thickness OD 0 440 (difficult reading) OS 0 454 Neurological examina

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CENTRAL CORNEAL THICKNESS IN LOW-TENSION GLAUCOMA

BY

NIELS EHLERS and FINN KRUSE HANSEN

Significantly reduced central corneal thickness is reported in seven patients with low tension glaucoma This finding together with previously reported findings of increased thickness in ocular hypertension *(suspicio glaucomatis)* illustrates the clinical importance of the corneal thickness in the evaluation of border line glaucoma

Key words glaucoma low tension – corneal thickness – pachometry – tonometry

With normotensive eyes a positive linear correlation exists between central corneal thickness and intraocular tension measured by applanation tonometry (Kruse Hansen 1971) Increased corneal thickness has been demonstrated in monosymptomatic ocular hypertension (Kruse Hansen & Ehlers 1971 Ehlers Kruse Hansen & Aasved 1974) and logically therefore a reduced thickness would be expected in low tension glaucoma This finding is reported below

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Methods

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Case histories

1 (AKH 300696) 6 year old man with visual field defects recognized for 2 years Treated with pilocarpine in spite of normal intraocular tension at all examinations Visual acuity OD 0 8 + 1 00 90 OS 0 6 + ? 00 65° Biomicroscopy was normal with open chamber angles Ophthalmoscopy showed glaucomatous cupping of the discs Perimetry revealed arcuate defects (Fig 1) Without treatment the intraocular tension was OD 16 mmHg OS 14 mmHg by applanation tonometry It was measured 3 times daily for 7 days and was never above ?0 mmHg A waterloading test showed no significant increase in pressure Indentation tonometry OD 5 5/5.5 g 10 0/10 0 g OS 5 0.5 5 g 10 0/10 0 g Central corneal thickness OD 0 43 mm OS 0 452 mm Neurological examination and plain *radiography of the skull with laminography of the sellar* region showed nothing abnormal EEG was normal Treatment with pilocarpine was continued

(AKH ? 0315) 58 year old woman complaining of headache Examination showed VOD 0 (–1 00 sph VOS 0 (7–0 50 sph Biomicroscopy including gonioscopy was normal ophthalmoscopy showed glaucomatous cupping of the discs Perimetry revealed arcuate defects (Fig 1) Applanation tonometry OD 14 mmHg OS 15 mmHg A waterloading test was negative Central corneal thickness OD 0 473 OS 0 478 mm Neurological examination and plain radiography of the skull showed nothing abnormal FEC was normal

3 (AKH 050501) ? year old man complaining of decreasing vision during one year Examination showed VOD hand movements in front of the eye VOS 0 5 emmetropia Biomicroscopy including gonioscopy was normal ophthalmoscopy showed glaucomatous cupping and degenerative changes in the macular regions Perimetry showed severe field defects (Fig 1) suggestive of late glaucoma Applanation tonometry OD 17 OS 18 mmHg Schiotz tonometry both eyes 4 0/5 5 g 9 0 10 0 g 13 0 15 g The applanation pressure measured several times daily during 6 days was never above 19 mmHg Waterloading test showed no increase in pressure but an epithelial oedema developed Central corneal thickness OD 0 440 (difficult reading) OS 0 454 Neurological examina

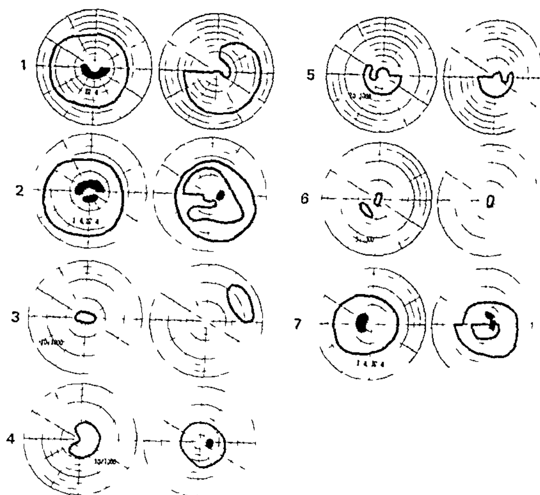


Fig 1
Visual field defects in patients with low tension glaucoma The numbers refer to the respective case histories

tion showed nothing abnormal Radiography of the skull revealed arteriosclerosis of the internal carotid arteries otherwise nothing abnormal EEG was normal

4 (KH 300/09) 64 year old woman complaining of headache The first examination 1963 showed VOD 1 0 + 0 5 sph VOS 0 1 no improvement with glasses Biomicroscopy was normal Ophthalmoscopy showed glaucomatous cupping of both discs (Fig 2) Perimetry of the right eye was normal but a glaucomatous defect could be demonstrated in the left eye (Fig 1) Intraocular tension was 18 mmHg in both eyes by applanation tonometry The intraocular tension was further measured 3 times daily for 4 days and was never above 18 mmHg Two waterloading tests showed no increase in intraocular tension that could be considered significant for glaucoma She was treated with pilocarpine.

The 1972 examination showed unaltered visual acuity in both eyes The intraocular tension was 15 mmHg by applanation tonometry in both eyes Perimetry showed almost



Fig 2
Glaucomatous cupping of the discs in case 4

unaltered defect Schiotz tonometry OD 5/5 5 g 8/7 g Central corneal thickness OD 0 466 mm

Plain radiography of the skull showed nothing abnormal Pneumoencephalography and left carotid angiography and EEG were normal

5 (KH 910410) 63 year old woman. A brother had glaucoma. The patient has always been myopic
The first examination in 1964 showed VOD 0.5–10 5 sph VOS 0 1–16 0 sph Biomicroscopy was normal Ophthalmoscopy showed myopic fundi The discs were excavated Perimetry showed large defects (Fig 1) Waterloading test was normal Intraocular tension was followed 3 times daily for 2 days and was always below 18 mmHg Plain radiography of the skull was normal EEG was normal In 19,9 the same visual acuity was found intraocular tension 18 mmHg both eyes Central corneal thickness OD 0 469 mm OS 0 468 mm

6 (KH 1,0717) 56 year old woman complaining of failing vision of the left eye during the past 6 months
The examination showed VOD 0 67–? 00 sph VOS 0 33–3 00 sph Biomicroscopy was normal Ophthalmoscopy *showed glaucomatous cupping of the discs Perimetry showed* large defects (Fig 1) Applanation tonometry 20 mmHg in both eyes Water loading test was normal The intraocular tension was measured 3 times daily and never exceeded 0 mmHg Central corneal thickness OD 0 467 mm OS 0 464 mm Plain radiography of the skull and EEG were normal

7 (AKH 1,0715) 59 year old woman operated twice for atoxic struma
Examination showed VOD 1 0 emmetropia, VOS 0 4–? 00 sph Biomicroscopy including gonioscopy was normal Ophthalmoscopy showed glaucomatous cupping of the discs the left was in addition malformed with abnormal arrangement of the vessels Perimetry revealed glaucomatous defects (Fig 1) Applanation tonometry 3 times daily for 11 days was always below 18 mmHg Water loading test normal Central corneal thickness OD 0 450 OS 0 45. mm Clinical neurological examination showed nothing abnormal Plain radiography of the skull left carotid angiography and EEG were normal

Corneal Thickness

Values for central corneal thickness and pressures* are shown in Table I. The average central corneal thickness in the seven patients was 0.461 ± 0.007 mm in the right eye and 0.466 ± 0.005 mm in the left eye. These figures are significantly lower than the normal values (see Table II, Kruse Hansen 1971) ($P < 0.001$). The average applanation pressure was 16.7 mmHg in right as well as left eye. None of the figures for applanation or indentation tonometry was above 20 mmHg.

Discussion

Patients with low tension glaucoma are not common, for which reason it was considered to be of interest to present these few cases. The visual field defects and excavated discs arouse the suspicion of glaucoma, but an intraocular pressure above 20 mmHg was never observed. Neurological examination and skull radiography were in all cases normal, excluding beyond reasonable doubt a neurological explanation of the findings.

Table II presents our findings of central corneal thickness in normal persons,

Table I
Data for 7 patients with low tension glaucoma

Case No	Corneal thickness mm		Applanation tonometry mmHg		Schiøtz tonometry mmHg	
	Right	Left	Right	Left	Right	Left
1	0.482	0.452	16	14	1[illegible]	1[illegible]
2	0.4[illegible]3	0.4[illegible]8	14	15	–	
3	0.44[illegible]	0.454	1[illegible]	18	20	0
4	0.466	–	15	[illegible]	1[illegible]	–
5	0.469	0.468	18	18	–	
6	0.46[illegible]	0.464	[illegible]0	20	–	
7	0.480	0.482	1[illegible]	1[illegible]	–	–
Average	0.461 ±0.0067	0.466 ±0.0050	1[illegible] ±0.[illegible]	1[illegible] ±0.81		

*Calibration Friedenwald 1955

Table II

Central corneal thickness in normal and glaucomatous eyes

	Normal		Glaucoma simplex		Suspected glaucoma				Low tension glaucoma	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
	*				*		***			
Average	0.570	0.574	0.5[illegible]1	0.526	0.543	0.557	0 566	0 575	0 461	0 466
s e m	0 00[illegible]	0 002	0 003	0 003	0 007	0 007	0 005	0 005	0 007	0 005
(no of cases)	16	14	16	14	95	93	8	8	7	6

Kruse Hansen 1971
Ehlers Kruse Hansen & Aasved 1974
Kruse Hansen & Ehlers 1971
* *Difference significant only at 1 % level*

in patients with glaucoma simplex in two series of patients with suspected glaucoma (ocular hypertension) and in low tension glaucoma There is no difference between central corneal thickness in normal persons and in patients with glaucoma simplex In the groups with ocular hypertension the corneal thickness is significantly above the values in normal eyes and in eyes with glaucoma simplex ($P < 0\ 001$ except for one group as indicated where the difference is significant only at the 1 % level) In our cases of low tension glaucoma the thickness is significantly below normal and glaucoma simplex values ($P < 0\ 001$)

In the presentation of the applanation tonometer (Goldmann 1954 Goldmann & Schmidt 1957) it was fully realized that the calibration would be influenced by the corneal thickness The Imbert Fick law $P = \frac{W}{A}$ (P = pressure in sphere W = applanating weight (force) and A = applanated area) ideally applies only to an infinitely small thickness The measuring conditions were so chosen that the corneal elasticity counteracting applanation, and the tear surface tension facilitating applanation balanced each other at the applanated area A corneal thickness of 0 5 mm was presumed It should therefore from the theory of applanation tonometry be assumed that variation in corneal thickness would have an influence upon the measured value for the pressure in the eye, in such a way that a thick cornea would give rise to an elevated reading a thin cornea to a reduced reading This has now been clinically supported In a future publication experimental data on pressure and thickness will be presented In the

calibration of the Schiotz tonometer, corneal thickness is not generally considered an important factor and deviations from ideality were collected under the designation "scleral rigidity" The reported data on indentation tonometry are too few to allow any comments

The present study has demonstrated a reduced corneal thickness in a group of patients with low tension glaucoma The thin cornea will tend to give a low reading for applanation pressure. It therefore seems possible that the intraocular fluid pressure in these eyes is above normal but that our tonometers are not calibrated for these particular eyes The finding of a thin cornea in low tension glaucoma is not supported by Tomlinson & Leighton (1973) who in a study of 11 cases of low tension glaucoma (applanation tension below 23 mmHg) matched with 11 cases of open angle glaucoma and 11 normal controls found the same average thickness in low tension glaucoma and normal controls but a larger thickness in open angle glaucoma The difference, however was not significant ($0.05 < P < 0.10$) Another explanation of the visual field defects and the cupping of the discs recently emphasized by Drance (1973) is an unusual vulnerability of the vascularization of the optic nerve head This and the thin cornea are alternative although not mutually exclusive explanations the relative significance of which awaits the study of larger series

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OPTICAL PACHYMETRY OF THE ANTERIOR CHAMBER

A methodological study of errors of measurement using Haag Streit 900 instruments

BY

P H ALSBIRK

In connection with a population study of the anterior chamber depth (ACD) measured optically with Haag Streit 900 instruments a field study of the method was performed
The *random error* of measurement was estimated in two ways a) on the basis of the triple readings of the measuring procedure s d = 0 020 mm and b) by re examinations after a few weeks in a random sample of 52 persons 0 03 mm i e. a significantly larger value. Corresponding results for corneal thickness were obtained (0 007 and 0 013 mm)
A highly significant *side difference* – with larger left eye values – was found in ACD (mean difference 0 070 mm) and in corneal thickness (*0 019* mm) in 900 pairs of eyes
The *correction values* due to radius of corneal curvature were found to be negligible.
Against a background of large interpersonal variations of ACD the methodological problems seem to be small ACD is a precise anatomical measure with this method

Key words optical pachymetry precision – errors of measurement – anterior chamber depth – corneal thickness

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In a population study of the anterior chamber depth (ACD) in Greenland Eskimos the Haag Streit 900 slit lamp attachments were used The two instruments – pachymeters – appeared in 1964 as a further development of a device introduced by Jaeger in 1952 Lowe (1966) found the pachymeters advantageous compared with earlier methods easy to handle to align and to read In ACD measurements a precision of about ± 0 05 mm was reported Correspondingly Weekers et al (1973) in a review of the method stated the precision to be 0 1 mm but without specifying whether standard deviation range or a confidence interval was used The method is being used increasingly in clinical work and population studies However a systematic analysis of random errors in ACD measurements performed with the Haag Streit pachymeters has apparently not been published so far

In order to obtain and control a satisfactory level of reliability during the population survey described elsewhere (Alsbirk 1974) the methodological problems had to be studied in the field As the findings are of general biometric interest in relation to pachymetry of the anterior segment a report is given below

Methods and Material

A General procedure of optical pachymetry in the population study of ACD in Eskimos

The instructions given by the manufacturers were strictly observed three readings following three alignments in about 20–40 sec were recorded in each eye with both pachymeters

The corneal thickness pachymeter (no I) was read to the nearest 0 01 mm and the distance between the front surfaces of cornea and lens was measured with pachymeter no II to the nearest 0 05 mm scale value

The mean value of each series of three readings was calculated and the proper internal ACD value was obtained subsequently as the difference between mean II and mean I

The slit lamp was transported in two ordinary trunks disassembled in eight parts A light wheeled table to which the original baseplate with transformer was fixed provided the only adjustment necessary In some villages electricity was supplied from a 6 volt motor car accumulator Reduced illumination of the room and the narrowest possible slit were used throughout The microscope was used with the smaller magnification objective (1x) in both pachymeters

B Consistency of the ACD measurements A study of random errors

A priori several sources of error might be expected to influence the results thus obtained According to recent terminology (e g Sokal & Rohlf 1969) the errors influencing physical measurements are of two different types 1) *Systematic errors* cause the measured values to deviate systematically from their true sizes thus affecting the *accuracy* 2) *Random errors* result in a dispersion of (repeated) measurements around the more or less accurate values thus affecting the *precision*

Generally no control of the accuracy was possible under the conditions of the survey The measurements had to be taken at face value As to the precision of the ACD measurements however the population survey was considered to give a favourable basis for a study As only one set of pachymeters was used and every measurement was made by me no interequipment or interobserver variation was involved

The precision of the method under these conditions was estimated in two different ways By the variation a) *within* the triple readings of a single measurement and b) *between* repeated measurements on different days

a) The variation of the three readings *within* each series furnished a pooled variance (s_w^2) with two degrees of freedom per person The error variance of the single measurements was calculated as $s_s^2 = 1/3 \times s_w$ for each pachymeter distance and $s_{s\,ACD}^2 = s_{s\,I} + s_{s\,II}^2$ for ACD

b) The difference (d) *between* independent results of the same eyes obtained with the interval of a few weeks furnished a supplementary estimate of the error variance of the single measurements However any short time fluctuation and day to day variation of the ACD level in the individuals might influence these estimates but not the almost momentary estimates mentioned above The conventional formula $s_b^2 \frac{\sum d^2}{2n}$ was used on all three distances with n = no of persons examined twice each contributing one degree of freedom

A random sample of 52 Eskimos aged 7–66 years was studied by these means, in a right eye and a left eye series

C Side difference in ACD and corneal thickness measurements

The pachymeters have to be used from the *left hand side* of the person examined with the slit image ocular exactly 40° from the visual axis of each eye. Thus the reading is made from the nasal side of the right eye and the temporal side of the left eye. The possible influence of this asymmetry of the method had to be checked Furthermore biological reasons called for a comparison of the two eyes, with an estimate of the intra eye pair variation

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The whole population sample from Umanaq furnished the material of the study. In 900/931 = 96.7 % of the persons examined both eyes were examined (Alsbirk 1974). The analysis of corneal thickness side differences comprised only 839 persons in whom no scarring was observed.

Later a supplementary survey was carried out (Alsbirk 1973) using ultrasound oculometry (Smith Kline Ekoline 12). A total of 599 pairs of eyes was measured in the adults of the previous sample. This completely different method is applied under symmetrical conditions in the two eyes. It thus afforded another chance of elucidating the possible side difference in ACD.

D. Pachymetry correction values according to radius of corneal curvature

Actually the value obtained with each of the pachymeters had to be corrected according to the conversion tables supplied with the instruments using the pachymeter reading and the radius of corneal curvature as entries. Generally, at least in a Caucasian population, the correction values should nearly always be negligible in eyes within a normal range (Lowe 1966, Weekers et al. 1973). However, in a population with unknown corneal radius distribution the possible importance of the correction values had to be studied.

With a Javal Schiøtz keratometer just calibrated the corneal radius was measured in both meridians and the average taken. The consulting ophthalmologist (V. Clemmesen) made the adjustments. The correction values were found in the two conversion tables and the final ACD correction obtained as the difference.

The material for this part of the study was 91 Eskimos selected at random in the adult town population of Umanaq, with 50 Danes from Umanaq as controls. Both eyes were measured.

Results

The results of the three studies outlined above are given in the Tables.

The random error of measurement are shown in Table I for each of the three distances. Very similar values were found in children aged 7–14 and in adults. As errors in right and left eyes were equal, average values are given. The comparable error variances were significantly larger between different days (b) than within the individual measurements (a) (e.g. $F_{101} = \frac{0.00138}{0.00040} = 3.45$ for ACD, $P < 0.01$).

Table I

Random errors of measurement in optical pachymetry with Haag Streit 900 instruments The error variance was estimated by *a*) variation within triple readings and *b*) differ ences *between* repeated measurements after a few weeks as average variance of eye pairs

Random sample of	23 children		29 adults		52 persons	
Distances measured (I & II) or calculated (ACD)	Variance mm	s d. mm	Variance mm	s d mm	Variance mm	s d mm
Corneal thickness (pachymeter I)						
a (s_{wI})	0 00005	0 007	0 00005	0 007	0 00005	0 00
b (s_{tI})	0 00017	0 013	0 00018	0 014	0 00018	0 013
ACD + corneal thickness (pachymeter II)						
a (s_{wII})	0 00037	0 019	0 00034	0 018	0 00035	0 019
b (s_{tII})	0 00107	0 033	0 0012?	0 036	0 00118	0 034
ACD (II–I)						
a ($s_{wI}+s_{wII}$)	0 0004?	0 021	0 00039	0 020	0 00040	0 020
b (s_t)	0 00147	0 038	0 001 ?	0 036	0 00139	0 037

Thus the ACD of a single eye was measured with an error s d of s_t = 0 037 mm corresponding to only 1 4 % of an average ACD value (2 61 mm) even if the day to day component of variation was included When the ACD value of a person was taken as the average of the eyes as was done in the population studies the corresponding error was 0 031 mm (empirically esti mated)

The side difference was found to be extremely significant with both pachy meters (Table II) No systematic age and sex variation appeared and pooled results are given The proper ACD mean difference was 0 020 mm i e only half the value of pachymeter II (0 039 mm) as the contribution from the corneal readings was relatively large (0 019 mm)

Thus in optical pachymetry the left eye mean values were significantly larger In ultrasound oculometry however no significant side difference appeared The standard deviation (s_D) was larger with this method ($F = 2\ 90$ $P < 0\ 01$)

The correction values showed the distributions given in Table III Only the mean value of female Eskimos differed significantly from zero – and from that of male Eskimos.

Table II

Side difference in optical pachymetry (D = left minus right eye value in mm) 900 pairs of eyes measured with Haag Streit instruments nos I and II Results of a subsample examined by ultrasound are included for comparison

	n	$\overline{D}$	s_D	$s_{\overline{D}}$	$t = \overline{D}/s_{\overline{D}}$	Significance	Correlation coefficient r
By optical pachymetry							
Cornea + ACD (II)	900	+ 0 039	0 083	0 0028	14 1	$P \ll 0\ 001$	0 98
Corneal thickness (I)	839	+ 0 019	0 018	0 0006	29 2	$P \ll 0\ 001$	0 84
ACD	900	+ 0 020	0 051	0 0027	7 5	$P \ll 0\ 001$	0 98
By ultrasound							
Cornea + ACD	599	− 0 008	0 138	0 006	1 42	n s	0 95

Table III

Correction values in optical pachymetry according to radius of corneal curvature

Sample	No of eye pairs n	Correction value of ACD (in mm)					
		x	s	$s_{\bar{x}}$	$t = \frac{x}{s}$	P	Range
Eskimos ♂	38	−0 006	0 070	0 003	1 72	n s	−0 05 → +0 03
Eskimos ♀	53	−0 025	0 070	0 003	9 2	< 0 001	−0 08 → +0 04
Danes ♂	24	+0 008	0 028	0 006	1 43	n s	−0 04 → +0 09
Danes ♀	26	−0 006	0 027	0 005	1 05	n s	−0 06 → +0 04

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ACD	900	+ 0 020	0 081	0 0027	[illegible] 5	$P \ll 0\ 001$	0 98
By ultrasound							
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Danes ♂	24	+0 009	0 078	0 006	1 43	n s	−0 04 → +0 09
Danes ♀	6	−0 006	0 077	0 003	1 05	n s	−0 06 → +0 01

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By ultrasound							
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indicated that certain day to day components of *variation must* be involved in the last one (b)

Bleeker (1963) studied the diurnal variation of ACD and pointed out that ACD *which indicates the* position of the ocular diaphragm is incessantly altering in normal eyes His observations were based on serial photographic recordings which often showed small but fairly synchronous fluctuations of ACD in both eyes of the examined persons Rosengren (1930) examined four eyes by 10 readings each series repeated 10 times (twice per day in 5 days) *He found the standard deviation between the 10 series to be almost double that* of the *within* series estimate very similar to my findings in Table I Bleeker (1960) seems to be right in pointing out that Rosengren overlooked the physiological fluctuations when he explained his findings solely as incorrect judgment by the observer *Physiological variation* must exist but how large? *Bleeker (1963) did not give the statistics of this problem* and with the small fluctuations involved a good estimate would be difficult to obtain

The present study was carried out in non cycloplegic eyes as was necessary in a population with the known tendency to angle closure From other studies it is known that various accommodative states influence the ACD (Calmettes et al *1958* Coleman *19 0* Delmarcelle & Luyckx Bacus *1971* Brown *19 3* among others) As regards Eskimos the cycloplegic changes in ACD were studied by Forsius (19 1) (who used cyclopentolate hydrochloride 1 % x 3 below 40 years and tropicamide 1 % with metaoxedrine 10 % over 40 years) The *non cycloplegic* ACD distributions were published by Alsbirk & Forsius (19 3) As the same Canadian and Alaska Eskimos were also measured in cycloplegia the considerable deepening effect of this state on ACD could be given see Table V (pooled data as no variation with age and sex was found) Therefore some of the physiological fluctuations influencing the *error estimate* (b) *have certainly* been due to accommodative changes

Table V

Deepening of the anterior chamber due to cycloplegia in 2 4 Eskimo right eyes Age range 9- 0 years median 9 years (Forsius 1971)

ACD increase class marks mm	0 00	0 05	0 10	0 15	0 20	0 5	0.30	0.35	0 40	0 0	Total
No of right eyes	44	53	6	33	45	20	6	1	4	1	2 4

Median 0 10 mm

Table IV
Variation components of ACD as encountered in a population study listed according to biological and methodological factors

Source of variation	Degrees of freedom	Mean square (mm^2)	S.D. (mm)
Total interpersonal variation	924	0.1648	0.41
Residual variation unexplained by age and sex	920	0.0847	0.29
Intra eye pair variation	900	0.0066	0.08
Random error of measurement (b)	52	0.0014	0.04
Random error of measurement (a)	104	0.0004	0.02
Correction values	59	0.0004	0.02

The radius of corneal curvature will be treated in a later paper: no ethnic but a significant sex difference was found (mean and s.d. for the Eskimos ♂ 7.90 ± 0.27 mm, ♀ 7.66 ± 0.25 mm).

As a background for the interpretation of the results concerning ACD the total variation pattern of the population study is summarized in Table IV. Consideration of age (< 15 & 15+) and sex according to four linear regressions (Alsbirk 1974) nearly halved the total variation (cf. the mean squares). In comparison the intra eye pair variation was found to be very small but still significantly larger than might be explained by the random errors of measurement.

Discussion

A penetrating analysis of all the error problems inherent in optical pachymetry was not within the scope of a field study like the present. However, the results did estimate some of the errors most relevant in practice.

The *random error of the optical ACD measurement* was studied rather comprehensively, because the precision of the applied method had not been reported in detail before. As shown in Table IV the random error variance was only 0.0014/0.0847 = 1.7 % of the variation which was not accounted for by age and sex. The significant difference between the two error estimates (a & b) moreover

eye) Correspondingly the Tyndall reflection of the optical corneal sections appeared as a rule to be more brilliantly glittering in the left eyes

Compared with my findings Delmarcelle & Luyckx Bacus (1971) and Weekers et al (1973) found a larger side difference (0 04 mm n = 299) Apparently they used pachymeter II only (and aligned *back of cornea* with front of lens) a procedure which probably augments the problem (cf Table II)

The findings of Tornquist (1953) using Stenstroms method agreed with these results with regard to the size and direction of the side difference (left eye deeper) – but all eyes were measured from the right side! This discrepancy is difficult to explain Tornquist reviewed the results given by Rosengren (1930) who measured all eyes symmetrically from the temporal side No side difference was found and Tornquist therefore suggested that his own result was due to a technical problem

After all a systematic error due to asymmetry of the measuring procedure positively correlated to the angle kappa seems to be inherent in Haag Streit 900 pachymetry However the practical consequences for a population study of ACD should not be large As Table II shows about 2/100 of a mm had to be added to an ACD right eye value in order to estimate the left eye value or vice versa In a corneal thickness survey the problem would be relatively larger

The average *correction values* were found to be of negligible importance even if a significant negative mean value (–0 025 mm) was found in the female Eskimos (Table III) To disregard this systematic trend has only resulted in a small underestimate of the sex difference observed (Alsbirk 1974) Further the variance was minimal (Table IV) The conclusion therefore appeared that the correction procedure – also in Eskimos – can be omitted in a population study

In summing up my results show that optical pachymetry of the anterior chamber depth is a precise method which is easily used wherever a slit lamp can work Although minor physiological and methodological fluctuations seem to occur the ACD value seems to be a remarkably reliable measure. A small side difference with greater left eye values is inherent in the method

Acknowledgements

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However, with regard to Table I it should *not* be concluded that physiological day to day variations accounted for the whole difference between the error variances (s_b and s_{w3}) The triple readings were taken in rapid succession using the pupil and iris surface as landmarks to secure axial centering Possible small changes in the use of these landmarks on different days would also influence the level of the readings The s_b^2 error estimates of ACD – as well as corneal thickness (cf Table I) – were generally 3 5 times greater than the s_{w3} estimates Thus undoubtedly both methodological and physiological factors inflated the s_b^2 values The relative importance of these factors eludes analysis but – most important – they add up to the s_b^2 values given The ACD error ($s_{b\,ACD}^2$) is very small compared with the total variation in the population (cf Table IV) Therefore the study seems also to show that the measuring procedure favours a uniform visual situation in which greater accommodative or other physiological changes hardly occur A 95 % confidence interval around an ACD value would be ± 0 07 mm while a ± 0 04 mm interval based only on s_{w3} estimates could not take the whole methodological error into account.

As for corneal thickness the ratio between random error and population variance was far less favourable $\frac{0\ 00018}{0\ 00106} = 17$ % Besides, the distribution of corneal thickness in the population will be described later (mean and s d for 839 right eyes 0 512 ± 0 032 mm)

The *study of the intra eye pair variation* revealed small but extremely significant *side differences* with both pachymeters It can be calculated that a sample of 107 eye pairs with regard to ACD and only seven as to corneal thickness had just indicated significance at the 0 01 level under the conditions given in Table II for $\overline{D}$ and s_D Was this phenomenon caused by technical or biological asymmetry? As indicated by the ultrasound data no real side difference seems to exist when a symmetric method is used

Possible explanations of the phenomenon have turned up from various sources When decentering pachymeter II horizontally Lowe (1966) stated that the right hand side appeared to be 0 15–0 20 mm deeper than the left hand side of both eyes of a person as a result of the varying obliquity of the light path through the corneal surface during decentering Ehlers & Kruse Hansen (1971) studying corneal thickness with pachymeter I demonstrated convincingly that the side difference (left cornea thicker) was positively correlated to the angle kappa as the measurement is performed in the visual not the optical axis of the eye In accordance with Lowe s statement concerning ACD they found the cornea to be about 0 07 mm thicker in the right side of an eye In an ordinary measurement of the left eye such a slight decentering towards the right will just occur the greater the larger the positive angle kappa (vice versa for the right

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THE c WAVE OF THE HUMAN D C REGISTERED ERG
I A QUANTITATIVE STUDY OF THE RELATIONSHIP BETWEEN c WAVE AMPLITUDE AND STIMULUS INTENSITY

BY

KLAS-OLAV SKOOG and SVEN ERIK G NILSSON

The *c* wave of the human ERG was studied with a new method, using d c amplification and averaging technique Stable and reproducible recordings of the *c* wave were obtained without the aid of general anaesthesia Within the investigated range of intensities (? log units) a linear relationship between *c* wave amplitude and log stimulus intensity was found This was valid whether the *c* wave was measured from the base line or from the bottom of the preceding trough The value of standardizing this method for routine clinical purposes is discussed

Key words electroretinography – clinical method – *c* wave – retina – pigment epithelium

The new method for d c registration of the human electroretinogram (ERG) at low and conventional stimulus intensities described by Knave Nilsson & Lunt (19 3) made it possible to obtain good recordings of the *c* wave component In the present study this method was further developed and now gives registrations

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ound in the literature concerning the presence of a *c* wave in recordings from retinas which have been separated from the eye cup In the papers cited below it s assumed that the pigment epithelium stays with the eye cup Using such iso ated frog retinas Sickel (1965 19/2) and Hohne (1971 1972 1973) recorded potentials which were similar to *c* waves However neither Yamashita (1959) nor Ames & Gurian (1963) could obtain *c* waves from the isolated toad or rabbit retina. Dowling & Ripps (19/2) recorded *c* waves from the intact aspartate treated skate eye but failed to do so from the isolated aspartate treated skate retina According to Rodieck (1972) the *c* wave arises when a large positive r potential from the pigment epithelium interacts with a large negative receptor potential In 19/2 Knave Moller & Persson published an analysis of the low intensity ERG of the sheep They proposed as ERG components a rod and a cone receptor potential a positive and a negative d c response and a slow late positive potential corresponding to the *c* wave at higher stimulus intensities A strong support for the opinion that the *c* wave originates in the pigment epi thelium was presented by Steinberg Schmidt & Brown (19/0) Intracellular recordings from pigment epithelial cells in the cat permitted the slow light evoked responses from these cells to be described in detail and identified positively as the origin of the *c* wave The pigment epithelial response seemed to be rod dependent Niemeyer (1973) later obtained similar responses from the pigment epithelium of the cat

The work cited above refers to animal experiments which yield reproducible recordings more easily than registrations from humans The first human ERG was reported by Dewar & McKendrick in 18/6 They obtained a crude electrical response on their galvanometer With great difficulties Kahn & Lowenstein (1924) Hartline (1925) Sachs (1929 1931) and Cooper Creed & Granit (1933) recorded human ERGs with a d c technique These authors do not state whether mydriatic drugs were used to eliminate iris potentials This makes it difficult to evaluate the *c* waves of their registrations Riggs (1941) and Karpe (1945) independently introduced the contact lens electrode and Karpe (1945 1948 1962) further developed the study of the *a* and *b* wave components of the electroretin gram into a clinical routine procedure Because of its slowness the *c* wave requires very stable conditions and a d c technique for its correct re gistration The standard clinical techniques for recording the comparatively fast *a* and *b* waves employ condenser coupled amplifiers and silver – silver chloride electrodes both of which are unsuitable for a true registration of the *c* wave Wirth (1951) although not using d c amplification, still demonstrated *c* waves which disappeared after short light adaptation and which could not be recorded following brief stimuli Dodt (1951) using silver – silver chloride electrodes studied the secondary rise of the human d c registered ERG He separated

which are stable and reproducible enough for quantitative studies of the human c-wave, also during very long experiments It seems feasible to develop it into a clinical procedure

The so called standing potential (SP) between the front and the back of the eye was first demonstrated by Du Bois Reymond (1849) It is considered to originate to a large extent in the pigment epithelium (Noell 1954, Heck & Papst 1957 Gouras 1969) which is a single layer of metabolically active cells between the choriocapillaris and the retinal receptor cells The changes in SP which follow the stimulation of the retina with light constitute the electroretinogram This phenomenon was first registered by Holmgren (1865) Because of the slowness of early galvanometers it was not until 1903 that a complete ERG was recorded by Gotch in the frog an initial small negative deflection followed by a comparatively fast positive wave and then a slow secondary rise Additionally an off effect was noted at the end of the stimulus These deflections correspond to the a- b-, c and d waves of our present nomenclature The signs a b and c were suggested by Einthoven & Jolly (1908) who also put forward a component analysis of the ERG Brucke & Garten (1907) reviewed early ERG work and showed the similarities between ERGs from different vertebrates They also stressed the importance of excluding iris potentials which may simulate c waves Granit (1933) separated the ERG into the processes P I P II and P III according to the order in which they disappeared during increasing ether anaesthesia in decerebrated cats Granit held that the c wave was mainly built up by P I although its form could be modified by the other processes He proposed that P I was a rod dependent process which more or less disappeared at light adaptation and which was not directly related to the firing of impulses in the optic nerve (1947) Noell (1954) suggested that the c wave originates in the pigment epithelium This layer was selectively destroyed by sodium iodate which also abolished the positive c wave leaving a potential of reversed polarity Brown & Wiesel (1961 b) recorded the local electroretinogram (LERG) in the cat with microelectrodes and found that the LERG equivalent to the c wave of the gross ERG reached a maximum close to the retinal side of the pigment epithelium They also believed that they recorded intracellular responses from the pigment epithelium with the same time course as that of the c wave but with opposite polarity Brown (1968) considered the c wave to be a rod dependent process He stated that the c wave must be small or absent in cone ERGs Gouras & Carr (1965) found a decrease in c wave amplitude when the central retinal artery was occluded in the monkey However they also reported signs of injury to the choroidal circulation with some photoreceptor loss which was seen in microscopic sections Thus it cannot be fully excluded that the reduced c waves might be explained by an impaired choroidal circulation Conflicting results are

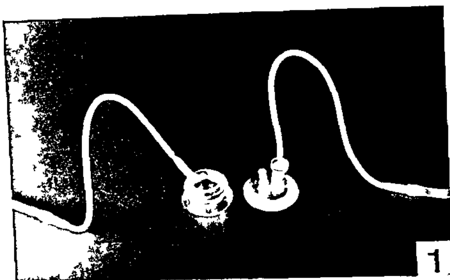


Fig 1
The scleral contact lens to the left and the plastic chamber for the forehead to the right The electrode tips are inserted in their holders.

scattering filter placed in front of the contact lens did not change the ERG recordings After cleaning the skin with alcohol a plastic chamber filled with Methocel® for the tip of the reference electrode was attached to the forehead with a piece of ring shaped two sided adhesive tape (Figs 1 and 2) If necessary a correcting lens corresponding to the volunteer s refraction was placed in front of the free eye. This eye was made to fix upon a very weak deep red light in the ceiling during each registration One of the arms was grounded The illumination of the eyes during the preparation procedure did not exceed 5 Lux Twenty minutes of further dark adaptation followed after the volunteer had been connected to the recording system

Recording system

Matched calomel half cells were used as recording and reference electrodes and they were connected to the contact lens and the plastic chamber by means of saline bridges in agar filled polyethylene tubes changed for each volunteer (Figs 2 and 3) The volunteer and the electrode system were shielded from alternating current etc by means of a wire net cage. The signals from the electrodes were fed into the differential inputs of a low drift d c amplifier

a pupillary and a retinal component. The latter, which nowadays would be called a *c*-wave, was found only after long dark adaptation and with long and strong stimuli. When the eye was repeatedly stimulated, the *c*-wave could in most cases be found only in the first ERG. During general anaesthesia prior to surgery a few d.c. recordings from human eyes were also made by Hanitzsch, Hommer & Bornschein (1966). With stimuli of high intensity and of long duration (several seconds) a distinct *c*-wave was demonstrated. Heilig, Thaler & Bornschein (1973) also used general anaesthesia to record slow components of the human ERG. They published two recordings, one normal and one pathological.

In the present study the method reported by Knave, Nilsson & Lunt (1973) was further developed in order to obtain even more reproducible conditions. For the first time, stable and reproducible recordings of the human *c*-wave are possible without the aid of general anaesthesia. A detailed analysis of the relationship between the stimulus intensity and the amplitude of the human *c*-wave was carried out. This analysis, as well as an investigation of the *c*-wave oscillations with time (Skoog & Nilsson 1974), were performed in order to map the normal behaviour of the *c*-wave and as a necessary basis for coming studies on the effect of drugs and other substances upon the retina and the pigment epithelium.

Material and Methods

The technique is based on the work by Knave, Nilsson & Lunt (1973) with several modifications.

Preparation of the volunteer

Three healthy volunteers, females aged 24 to 26 years, were chosen. They were not under the influence of drugs or stimulants. Both pupils were dilated to 8 mm or more with 0.5 % tropicamide and 10 % metaoxedrine given topically. The volunteer was kept in darkness for at least 30 min. Then, after topical tetracaine anaesthesia, a scleral contact lens (for the tip of the recording electrode) (Fig. 1) filled with Methocel® (Baeschlin) was applied to one of the eyes (Fig. 2). Compared to the lens of Knave, Nilsson & Lunt (1973) the present lens system was improved by changing the angle of insertion of the tube holder and by using a more pliable tube (Silastic®) close to the lens. The second channel of the earlier lenses was omitted. The lens was slightly opaque in order to give a uniform wide angle illumination of the retina. An extra thin opaque light

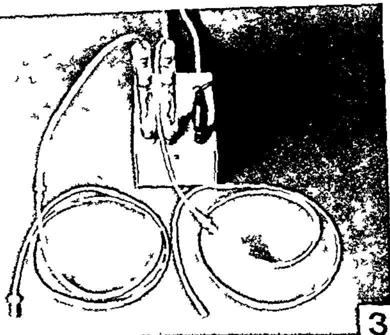


Fig 3

Matched calomel half cells were used as recording and reference electrodes The electrode tips were connected to the half cells by means of saline bridges in agar filled polyethylene tubes

intensities of 3 5 4 0 4 5 5 0 and 5 5 log units above the *b* wave threshold were used The flash interval was 15 sec at log rel intens 3.5 and 4 0 but 30 sec at log rel intens 4.5 and 5 0 Four responses were averaged at log rel intens 3 5 and 4 0 Two responses were averaged at log rel intens 4 5 and 5 0 whereas single recordings were used at log rel intens 5 5 The stimulus light was led to the eye through a quartz fibre optics (Schott) At the end of the fibre optics a centering ring was attached allowing a correct positioning of the light beam at a standard distance of 30 mm from the cornea in total darkness (Fig 4) Since the light beam itself was not needed to centre the fibre optics dark adaptation was not disturbed This was a modification of the earlier method which was also changed by using an adjustable helmet as a holder for the fibre optics (Fig 4)

In all 15 series of registrations (4–6 per volunteer) each consisting of responses with stimulus intensities from 3.5 to 5.5 rel log units were carried out.



Fig 2
The contact lens and the plastic chamber applied

They were lowpass filtered (220 Hz cut off 18 dB/octave) before they reached a Hewlett Packard signal analyzer 5480 S either directly or after having been displayed in a digital buffer connected to an oscilloscope This buffer which was an addition to the earlier method made it possible to discard those very few recordings which were disturbed by large eye movements The noise level of the electrode system was 5–10 μV and the d c drift 10–15 μV/h

Stimulus light

For light stimulation a xenon lamp (Osram XBO 150 Watt ozone free) with an approximately flat spectral emission curve within the visible part of the spectrum was employed The beam passed heat reflection and heat absorbing filters (Zeiss) Neutral density filters (Balzer) were used to change the light intensity The lowest intensity eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log relative intensity 0 The stimulus duration was kept at 1 sec by means of an electromagnetic shutter (Zeiss) Relative stimulus

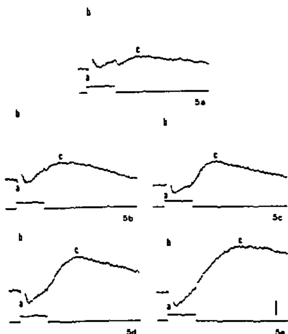


Fig 5
The d c recorded human ERG in response to stimulus intensities of a 3.5 b 4 0 c 4.5 d 5 0 e 5 5 rel log units above the *b* wave threshold Stimulus duration 1 0 sec, indicated on lower line Amplitude calibration 100 μV

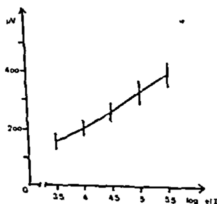


Fig 6
The relationship between *c* wave amplitude, measured from the base line and log rel stimulus intensity (Means and standard error of the mean from 15 experiments)

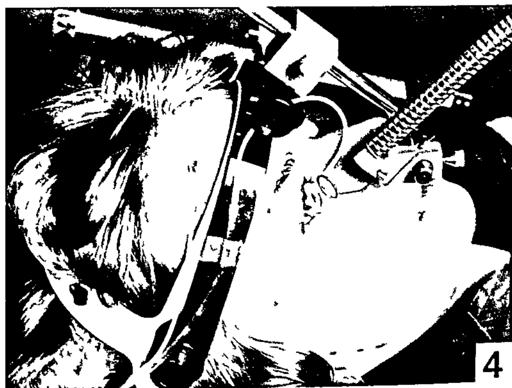


Fig 4
The fibre optics and its holder attached to the volunteer

One series of registrations lasted 5 min A pause of 2–3 min was made between the series

Results

A series of d c registrations of the human ERG with rel stimulus intensities ranging from 3 5 to 5 5 log units above the *b* wave threshold is shown in Fig 5 Stimulus duration 1 sec In addition to the *a* and *b* waves a *c* wave and a *d* wave (off effect at the end of the stimulus) are also found The *d* wave was most prominent in recordings with low stimulus intensity As is easily seen the *c* wave increased with increasing stimulus intensity Thus Fig 5 c demonstrates a very large and predominant *c* wave

In Fig 6 *c* wave amplitude as measured from the base line is plotted against log stimulus intensity (Figs 6 and 7 show means with standard error of the mean from 15 experiments) In Fig 7 the *c* wave amplitude was measured

Preliminary test clearly showed that the *c* wave amplitude did not change as a result of immediate light adaptation with the stimulus intervals used There was no tendency for the subsequent *c* waves to be smaller than the first one at each intensity step This was controlled by means of the buffer display unit before the signal was sent to the signal analyzer for averaging However the *b* wave amplitudes (measured from the trough of the *a* wave) tended to decrease somewhat at high stimulus intensities Thus the *b* wave was probably slightly affected by light adaptation The stimulus intervals used allowed the ERG curve to return to the base line after the *c* wave before the next flash was delivered This was noted on the oscilloscope during preliminary experiments Prior to every stimulation the stability of the base line was controlled by means of a sensitive galvanometer

Experiments on sheep (Calissendorff Knave & Persson 1974) and man (Skoog & Nilsson 1974) demonstrated that the *c* wave amplitude changed in an oscillatory manner in response to repetitive stimulations The frequency of these oscillations was about 2/hour and the change was generally damped In order to avoid disturbing interference from these oscillations each series of intensity amplitude registrations was made as short as possible although some averaging was performed Since the series each lasting 5 min were distributed on different phases of the oscillations the influence of the cyclic variations was kept under full control There were also indications of even slower oscillations of the *c* wave amplitude (Calissendorff Knave & Persson 1974 Skoog & Nilsson 1974) but because of their very slow time course they would not measurably change the relationship between the different *c* wave amplitudes in our series of recordings

Many authors have investigated the relationship between the stimulus intensity and the size of ERG components Dewar & McKendrick (1876) reported that the amplitude of their recordings presumably corresponding to the *b* wave was proportional to the logarithm of stimulus light intensity within a range of 2 log units Working with frog eyes De Haas (1903) also obtained such a relationship except at low stimulus intensities Brossa & Kohlrausch (1913) reported that all ERG components grew with increasing stimulus light. Hartline (1925) plotted the sum of the *a* and *b* wave amplitudes (ordinate) against log stimulus intensity (abscissa) obtaining an S shaped curve which tended to run parallel to the abscissa at low and at *high intensities Similar findings* were reported by Muller Limmroth (1953 1959) Wirth & Zetterstrom (1954) Asher (1960) and others According to Muller Limmroth the curve which describes the relationship between the *c* wave amplitude and log stimulus intensity is S shaped This was found in experiments with isolated frog eyes Other animal recordings have shown that the *c* wave increases with rising stimulus intensities at dark adaptation whereas it disappears at light adaptation (Einthoven & Jolly 1908 Brossa

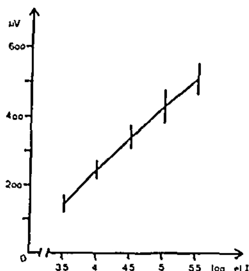


Fig 7
The relationship between *c* wave amplitude measured from the bottom of the preceding trough and log rel stimulus intensity (Means and standard error of the mean from 15 experiments)

instead from the bottom of the preceding trough Within the range studied a linear relationship between *c* wave amplitude and log stimulus intensity was found in both cases Also the amplitude of the trough preceding the *c* wave was linearly related to log stimulus intensity The implicit time of the *c* wave tended to be longer with increasing stimulus intensities which can be seen in Fig 5

The *a* wave amplitude increased steadily with increasing stimulus intensity The *b* wave amplitude measured from the trough of the *a* wave was fairly constant tending to be slightly smaller at both ends than at the middle of the intensity range

Discussion

The methodological modifications described above aiming at minimizing the disturbance of the dark adaptation during the preparation of the volunteer allowing a more exact positioning of the eyes as well as of the contact lens and the stimulus light beam during each registration and eliminating the few recordings distorted by large eye movements proved to be quite important to further stabilizing the system The intensity range used was chosen because flashes stronger than log rel intensity 5 5 often induced disturbing eye movements whereas flashes weaker than log rel intensity 3 5 resulted in very small *c* waves which were difficult to measure

defective interaction between the receptors and the pigment epithelium It should be interesting to look for changes in c wave shape and amplitude *in other diseases of the retina* and the pigment epithelium Our present method for the first time gives stable and reproducible recordings of the human c wave without the aid of general anaesthesia It is well tolerated also by untrained subjects A standardization for routine clinical purposes now has to be done taking into consideration also the c wave oscillations with time (Skoog & Nilsson 1974)

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& Kohlrausch 1915 Granit 1933 1947 Brown & Wiesel 1961 a Brown 1968 Wündsch & Bornschein 1972 Knave Moller & Persson 1972 and others) Schmidt & Steinberg (1971) demonstrated a linear relationship between log stimulus intensity and the amplitude of intracellular responses from the pigment epithelium of the cat after short flashes of light (480 msec)

Because of technical difficulties or for other reasons no quantitative studies of the *c* wave amplitude in relation to different stimulus parameters have been performed on humans To a certain extent Wirth (1951) Dodt (1951) and Hanitzsch Hommer & Bornschein (1966) noted characteristics of the human *c* wave similar to those found in animals

Our registrations showed a linear relationship between log stimulus intensity and *c* wave amplitude within a range of 2 log units This was valid whether the *c* wave was measured from the base line or from the bottom of the preceding trough Flashes of light with a duration of one sec and a rel intensity of 4 5 log units above the *b* wave threshold produced pronounced *c* waves and were easily tolerated not only by the volunteers but also by untrained patients Thus it seems possible to develop this method into a clinical routine procedure.

As mentioned above the question of the origin of the *c* wave is still under some discussion Using isolated retinas some workers (Sickel 1965 1972 Hohne 1971 1972 1973) obtained potentials which looked like *c* waves Other authors (Yamashita 1959 Ames & Gurian 1963) did not find such potentials These retinas were removed from the eyecup presumably leaving the pigment epithelium behind However to our knowledge no electron microscopy was done to prove the absence of pigment epithelium remnants The experiments by Steinberg Schmidt & Brown (1970) and Schmidt & Steinberg (1971) very convincingly correlate intracellular responses from pigment epithelial cells with the *c* wave of the gross ERG Similar responses although only for very short moments were found by Brown & Wiesel (1961 b) The work of Steinberg et al was supported by findings in connection with other intracellular recordings from the pigment epithelium (Niemeyer 1973) Noell (1954) and Nilsson Knave & Persson (1974) showed how the destruction of the pigment epithelium was accompanied by the disappearance of the *c* wave These data suggest that a gross destruction of the pigment epithelium in pathological conditions ought to be reflected as an alteration of the *c* wave A recent report by Heilig Thaler & Bornschein (1973) showed two d c recordings of slow potentials of the human ERG obtained during general anaesthesia One of their registrations was quite normal with a distinct *c* wave The other recorded from a patient with hemeralopia congenita lacked the *c* wave Instead a slow negative potential was seen The authors stated that the pigment epithelium looked normal in this condition but they suggested that the *c* wave might be absent because of a

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The inadequate text and lack of arrows and other reference signs makes interpretation of the illustrations difficult for the inexperienced. There are also technical abbreviations which are used without being defined.

The atlas consists mainly of illustrations of biopsy specimens, arranged in topographical sections starting with the palpebrae and going backwards to the nervus opticus *and orbita with the extraocular muscles. There are also a large number of pathological* cases – rather randomly chosen, it would seem – but there are several of importance as well.

The many sources which this atlas has drawn on have naturally caused it to vary considerably in quality. Both postmortem and optimally fixated material has been used. One major shortcoming is that there are so few light microscopic illustrations to compare with the electron microscopical sections and to indicate their origin.

Whilst this atlas will undoubtably be of use to practising electronmicroscopists as a *reference book to compare against their own material, it will hardly fullfil* the editor's hope that ophthalmologists in everyday practice will refer to it for a better insight into the mechanisms of basic diseases.

O. I. Jensen

Rossman Hermann Augenheilkunde Studienbuch fur Krankenschwestern Krankenpfleger und medizinisch technische Assistentinnen Ophthalmology Textbook for Nurses and Technical Assistents Kohlhammer Stuttgart 1972 86 pages Price DM 17 50

In 86 pages and in German the author presents an excellent and instructive book for nurses and technical personnel employed in ophthalmological departments It should never be forgotten by those in charge of ophthalmological departments that theoretical learning should be accompanied by practical instruction The education of our technical personnel forms a real problem and needs formalising

P Brændstrup

Wright Thomson A M The Life and Times of Dr William Mackenzie Privately printed by Robert Maclehose and Company Limited the University Press Glasgow 1973 Price

In 1974 the Glasgow Eye Infirmary is celebrating its 150th anniversary As William Mackenzie (1791–1868) was responsible for its foundation it is most appropriate that this delightful biography is now available

Apart from local Scottish colour the author presents many details of Mackenzie s life and activities and also his cultural and professional background

Mackenzie s main contribution to ophthalmology was *A Treatise on the Diseases of the Eye* the first English edition appeared in 1830 and the fourth in 1854 The first edition also found its way to Denmark and for me has been impressive reading – when one considers that without an ophthalmoscope and our present refined methods of examination his clever mind could sort out and intuitively deduct a logical understanding of many aspects of ocular affections

The greater part of the biography deals with Mackenzie s younger life with his Grand Tour leading to his final decision to take up ophthalmology

Extensive comments to this *Treatise* are presented

All of us have cause to appreciate Mackenzie s pioneer work – and that of the biographer

P Brændstrup

Yamada E & Shikano S I Atlas de Microscopie Electronique en Ophthalmologie Atlas de Microscopia Electronica en Oftalmologia Atlas di microscopia Elettronica in Oftalmologia Doin Paris 1973 376 pages 185 illustrations (21 × 29 7 cm – 2 250 g) Price F Fr 360 00

This 2 25 kg atlas consists chiefly of electron micrographs taken from Japanese ophthalmological journals and a few original illustrations by one of the editors Yamada Each right hand page has a reproduction of an electron micrograph the left hand page being reserved for the text which is much too brief Often only three or four lines are given leaving the rest of the page blank which strikes one as odd in view of the world s limited resources The English edition which I have seen looks even blanker than the tri lingual edition being reviewed here Furthermore one also wonders whether it was really necessary to translate into two so similar languages as Spanish and Italian

Princess Margaret Hospital Swindon Royal Army Education Corps and Royal Military College of Science Swindon England

ULTRA VIOLET RADIATION AS A POSSIBLE CAUSE OF CORNEAL DEGENERATIVE CHANGES UNDER CERTAIN PHYSIOGRAPHIC CONDITIONS

BY

F C RODGER J A CUTHILL, P J FYDELOR and A P LENHAM

Various geophysical factors have been blamed as the cause of a rare blinding disease found in the Red Sea littora A small expedition of the Scientific Exploration Society backed by The Royal Military College of Science in England whose laboratories were utilised visited the Dahlak group of islands There it was discovered that in some areas human eyes are subjected to relatively high short ultra violet radiation (SUVR) doses This was true not only for total integrated ultra violet radiation but also in the case of that proportion reflected from white coral sand especially when contaminated with salt The day long activities of the inhabitants whose living depends on diving and fishing in islands without shade as well as the weather conditions contribute to a complexity of circumstantial evidence strongly supporting the view that the condition known as climatic keratopathy arises from the cumulative effect on the exposed part of corneal e y e lly in the range discovered there – 290 to 3 0 nm Laboratory work was carried out on samples of topsoil on the islands and in the mainland of Eritrea Ultra violet sensors and detectors were used under different circumstances to augment the information This paper amplifies the clinical paper on Dahlak blindness published in the British Journal of Ophthalmology

Key words corneal ultraviolet radiation – geographical ophthalmology Dahlak Islands.

Received April .. 1974

Oxford Symposium

A symposium on The Eye in the Inborn Errors of Metabolism will take place at Oxford University Oxford England from April 13 to 16 1975 Speakers will include Norman Ashton Elmer Ballintine Elaine Berman Anthony Bron Donald Bergsma Eliot Berson Robert P Burns Ronald L Carr David G Cogan Patrick I Condon Edward Cotlier Harold Cross Glyn Dawson Monte A Del Monte Albert Th Franceschetti Jules Francois Alec Garner Morton F Goldberg Brian Harcourt William F Hughes Barry Jay Kenneth Kenyon Gordon Klintworth Toichiro Kuwabara Irene Hussels Maumenee Michael D Sanders Jack D Singer George Spaeth C Takki Ramesh Tripathi Ruth van Heyningen J M Walshe Warren Wilson Jonathan Wirschafter Vernon Wong Wolfgang Zeman A limited number of free papers will be accepted Ophthalmologists paediatricians or basic scientists are invited to attend register in advance For information regarding registration or participation contact the symposium organisers Edward Cotlier MD at the University of Illinois Eye and Ear Infirmary 1855 W Taylor Street Chicago Illinois 60612 or Anthony Bron FRCS University of Oxford Nuffield Laboratory of Ophthalmology Walton Street Oxford OX2 6AN England

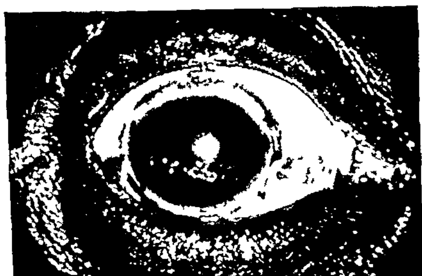


Fig 1
Bietti s corneal degeneration – nodules in exposed area of cornea

protection against ultra violet light in Spring The Dahlak i landers having a less strong visible and SUVR environment do not wear any protection and so in the long term their eyes could be more vulnerable

A subject responds to visible radiation but does not directly sense the accompanying ultra violet Since the latter is very strongly scattered by the environment the irradiation of the surface of the cornea from all directions is not directly related to the brightness of the scene viewed and the visual discomfort is not therefore a useful monitor of the SUVR dosage at the cornea

Table I
Proporti ns of bl nding diseases among
Dahlak islanders

B etti s corneal degeneration	5 0 per cent
Cataract	31 0 per cent
Unkn wn orig n	10 0 per cent
Other	[illegible] 0 per cent
	100 0 per cent

The work reported in this paper was undertaken as a step in the elucidation of the causes of a curious corneal degeneration (Bietti s corneal dystrophy climatic keratopathy) reported as widespread in the Dahlak Islands in the Red Sea with the ultimate objective of devising means of alleviating or eliminating the malady

Various suggestions of causation by climatic conditions had been made (Zanettin 1937 Falcone 1954 Bietti Guerra & de Gaspare 1955) To investigate the clinical symptoms at first hand and to probe the causation a small expedition (F C R and J A C) was mounted to the Islands for a period of 5 weeks in November December 1971 Owing to the relative inaccessibility of the Islands and the primitive conditions there only simple man portable equipment could be taken

Since ultra violet irradiation had been suggested as the most likely causative agent (Bietti et al 1955) photometric equipment was provided by the Royal Military College of Science Shrivenham (RMCS)

Due to transport difficulties beyond the control of the expedition some equipment arrived late and in consequence the photometric measurements were not as extensive as originally planned but significant information was obtained which seems to bring much closer the solution to the problem This was supplemented by measurements made subsequently at RMCS on samples of surface material collected by the expedition

The clinical picture

The results of the clinical examinations have already been reported (Rodger 1973) nearly 60 % of the total population are blind over half from climatic keratopathy and 45 % of all islanders examined exhibited some stage of this same corneal disease (Table I) In the advanced lesion the damage was most severe over the bi convex region left unprotected by nearly closed lids (Fig 1) The development to total blindness seemed to be slow 20–40 years There were strong resemblances in the cytoarchitecture of the degeneration to those found in cases of radiation damage to skin It is hoped to publish these findings shortly

It is known that in the region of the spectrum from 290 to 320 nm SUVR some 90 % of the radiation is absorbed by the corneal stroma and the rest by the lens In a strong SUVR environment such as a sunlit snowfield with a clean atmosphere at a high altitude the unprotected corneal epithelium is damaged after a few hours exposure This is comparable to what occurs in exposure to industrial welding and Rodger (1973) found a woman patient on one of the islands (Dohul) with identical clinical changes in both eyes As Forsius (1972) has pointed out circumpolar populations have long worn slit goggles as a



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Table 1
Proportions of blinding diseases among Dahlak islanders

Bietti s corneal degeneration	57 0 per cent
Cataract	31 0 per cent
Unknown origin	10 0 per cent
Other	2 0 per cent
	100 0 per cent

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Time integrated dosages of total ultra violet In order to measure the integrated SUVR (direct scattered reflected) received over a period of several weeks eye level vertical south facing photosensitive plaques were set up in four locations on Dahlak Kebir and the nearby mainland

Identical plaques were set up at RMCS and exposed to the equivalent of 9 days sunshine The midday airmass (pathlength of incident solar radiation in the atmosphere compared with that if the sun was directly overhead) at RMCS was 1 47 compared with 1 24 at Dahlak Kebir

The PVC (polyvinyl chloride) sensors used respond strongly to the wave lengths 290–310 nm and less strongly to 380–4[illegible]0 nm Sensors exposed at RMCS showed little colouration The colouration of the expedition sensors was pro nounced

The HX (specialist polymethyl metacrylate) detectors show photochemically produced absorbance at 340 nm after irradiation by SUVR This absorbance increases linearly with integrated dosage of SUVR The measured absorbances were

Site	Location	Duration of exposure (days)
1	Massawa (mainland shoreline)	21
2	Dahlak Kebir (over water)	21
3	Dahlak Kebir ([illegible]00 m inshore)	[illegible]1
4	Dahlak Kebir ([illegible] km inland)	16

Site	Absorbance	Exposure (days)
1	0 4	1
2 & 3	0 11	21
4	0 31	16
RMCS	0 04	9

Ultra violet dosages

We are concerned with the total integrated SUVR from primary and secondary sources received at the front centre of the eye by a subject in a working attitude In the present case this may be considered to be the total SUVR incident on a vertical plane The contribution of scattered light – especially that diffusely reflected from the ground – is important (Robinson 1963 Urbach 1969) The incident total SUVR can only be found by *in situ* field measurements using plane detectors

The Dahlak Islanders work from dawn to dusk in the open in a flat terrain without natural or artificial shade The sea winds readily evaporate body perspiration enabling them to work longer hours in strong sunlight than is possible on the mainland (Fig 2)

Meteorological data for the Islands are scanty but there is prolonged sunshine outside the rainy season and very good visibility for 90 % of the year (Fantoli 1966) so little impedance of SUVR from aerosols or dust occurs

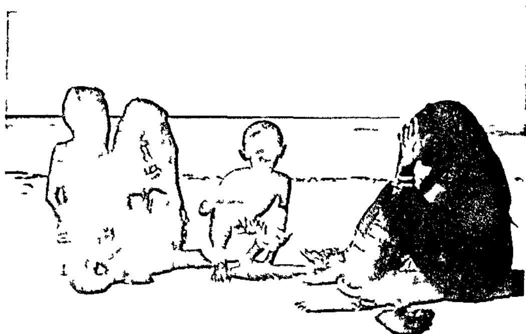


Fig 2
Dahlak Island women and children watching fishtraps on a sand spit above a coral undersea shelf

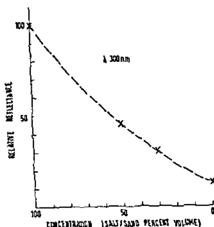


Fig 3
Reflectance at 300 nm of Red Sea Salt and Dahlak Coral sand concentrates relative to natural salt from the salt pans

Discussion

The inhabitants of the Dahlak Islands are healthy and not inbred It thus appears unlikely that the blinding disease they are subject to is hereditary

The typical level of short ultra violet irradiation is high although not dramatically so but scattered radiation in some of the Island situations appears to make a rather high contribution to the total dosage The laboratory tests suggest reflected SUVR will also be high on salt splashed beaches The main feature peculiar to the islands is the unremitting exposure This arises from the almost entire lack of shade and the fact that economic pressure and the amelioration of thermal stress by cooling breezes cause the men and women to be exposed on the beaches and other situations at work for long periods in their native occupations which are mostly connected with the sea diving and fishing and drying fish on the beaches

The correlation between the severity of corneal degeneration and the age of the islander (Rodger 19 3 Fig 6) implies a blinding mechanism that is cumulative with time as would be expected if the damage was caused by exposure to the environment.

It is unfortunate that air sampling equipment supplied by Porton Down was lost in transit However no clinical evidence was found of significant damage by foreign matter to eyes in the early stage of degeneration It was only in

Correction for exposure and airmass raises the RMCS value to approx 0 1 for 21 days exposure Sites 1 and 4 show very high dose rates It follows that at least in some situations in the Red Sea area human eyes are subject to relatively high SUVR dosages Site 3 was set over dark laterite rock and sand site 2 over water which reflects little SUVR This suggests that diffusely reflected radiation is highly important

Direct and indirect irradiations To obtain some information regarding the relative importance of direct and scattered radiation in the total dosage a simple photometer incorporating a plane vertical detector was used This was held at eye level and measurements were made in four directions upsun (obtaining direct solar radiation plus forward scattered radiation) downsun (back scattered radiation) sidesun (90° from the sun with scattered radiation predominating) Measurements were taken at sites 1 and 2 and for comparison at RMCS over grassland and at similar solar altitudes during an anticyclonic spell

These observations indicate the high amounts of scattered radiation at site 1 The very high overall dosage at site 1 confirms the results obtained with the integrating plaques

Reflection by surface materials The diffuse reflectance of a number of surface materials was measured at 300 nm The diffuse reflectivities of soils and coral sands varied from 3 to 9 % Salt from the salt pans was much more reflective (60 %) The expedition frequently noted salt splashes on soil surfaces especially on the shoreline The scattered light contributed by surface reflection is obviously greatest over salt rich terrain as was demonstrated in the laboratory (RMCS) with the samples brought back from the Dahlak Islands (Fig 5) it probably plays a key role

Except at small angles of incidence and reflection the contribution from SUVR reflected by the sea is low and unimportant

Site	Intensity		
	Upsun	Downsun	Sidesun
1	130	44	37
2	74	30	35
RMCS	111	22	37

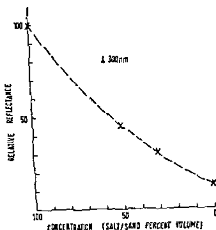


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Except at small angles of incidence and reflection the contribution from SUVR reflected by the sea is low and unimportant

Site	Intensity		
	Upsun	Downsun	Sidesun
1	150	44	37
2	74	50	95
RMCS	111	22	87

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severely damaged eyes (which have become anaesthetic) that embedded material was found Corneal degeneration leads ultimately to a lack of sensitivity to surface damage while in the early stages the healthy eye responds quickly to the arrival of foreign matter Although there is no evidence of wind blown material initiating the disease it is quite possible that it may play a part in the later stages of degeneration

The response of Dahlak Islanders to the sunlight was normal in that the eyes were narrowly closed when looking towards the sun The subjects reactions are conditioned mainly by the visible radiation received from a small solid angle covering the central vision whereas it is the direct and scattered radiation from almost the whole of the half hemisphere towards which the eye is facing that is responsible for the irradiation of the exposed cornea which contains unlike skin neither keratin nor melanin to impede the penetration of SUVR

The narrow bi convex shape of the severely damaged areas of cornea is strongly suggestive of irradiation produced damage, as is oedema of the lids described in the clinical paper by Rodger (1973)

Unfortunately no information is available regarding the integrated ultra violet dosages encountered by the inhabitants in other areas where similar corneal degenerations have been reported (see Forsius 1972 and Freedman 1973)

Environmental SUVR levels shade and work habits (exposure times) must be studied in comparing different locations Comparative studies of this nature would clarify the role of the SUVR

With the long time scales involved experiments with animals (whose life spans are invariably shorter than man s) are rather impractical so more field studies appear to be necessary if an early solution is to be found

Conclusion

There is some evidence that prolonged exposure to short ultra violet radiation may cause the blinding disease found in the Dahlak Islands The provision of simple eye protectors may therefore prevent this disease

Acknowledgement

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in order to investigate the nature and incidence of the various microorganisms harbouring on the normal conjunctiva (Matafune & Albanese 1912 Lundsgaard 1923 McKee 1924 Lucic 1927 Keilty 1930 Gowen 1934 Khorazo & Thompson 1935 Rodin 1945 Barfoed 1953 Smith 1954 Soudakoff 1954 Cason & Winkler 1954 Chang 1957 Orfila & Courden 1961 de Ocampo et al 1965 Glawogger 1969 Makabe 1971 Locatcher Khorazo & Seegal 1972) or to study the pro phylaxis of postoperative infections (Liebermann & Lengyel 1911 Lowenstein 1911 Imre 1911 Kraupa 1914 Betts 1921 Stanka 1924 Elshing 1926 Kuffler & Schneider 1926 Edmund 1927 v Pellathy 1932 Locatcher Khorazo 1953 Doden 1957 Zetzsche 1962 Drewnoik & Wachtel 1967) or lastly to answer the question of the value of bacterial cultures prior to intraocular surgery (Lindner 1914 Cooper 1935 Dunnington & Locatcher Khorazo 1945 Nolan 1967, Allan smith et al 1969)

The bacterial flora of the normal conjunctiva seem to be well defined *Staphylococcus albus* and *corynebacteria* are most frequently found and many other organisms including *Staphylococcus aureus* gram negative bacteria and streptococci occur in varying percentages of cases studied (Axenfeld 1907) This statement was later confirmed by most of the above mentioned studies However our understanding of the topographical distribution of bacteria on the normal conjunctiva still seems to be somewhat unclear Eyre (1898) claimed that the upper fornix was much more frequently sterile than the lower Pillat (1921) found that *S albus* occurred mostly on the upper palpebral and fornical conjunctiva in contrast to pneumo streptococci which were mainly localised on the bulbar regions Corynebacteria were found in equal quantities on the various parts of the conjunctiva One of these findings was latter confirmed by Gowen (1934) who found that *S albus* harboured mainly on the upper palpebral con junctiva Locatcher Khorazo & Seegal (1972) stated that the number of bacteria recovered from the upper fornix were often larger than that found on the lower

The present investigation was undertaken primarily in order to shed more light on this problem i e to study the topography of bacteria localised on the normal conjunctiva Furthermore to determine the immediate changes which may occur on topography after the instillation of anaesthetic eye drops (0 2 % benoxinate hydrochloride) into the eye

Material and Methods

The mater al comprises 100 patients selected from the out patients of the Eye Depart ment of *Kommunehospitalet* It was made certain that none of the patients showed any sign of eye inflammation or misfunction of the lacrimal apparatus that none of them

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BACTERIAL FLORA OF THE NORMAL CONJUNCTIVA
I TOPOGRAPHICAL DISTRIBUTION

BY

J A FAHMY* S MOLLER** and M WEIS BENTZON**

The bacterial flora of 100 normal conjunctivas was studied *Staphylococcus albus* and corynebacteria were the most common microorganisms recovered followed by *Staphylococcus aureus* gram negative bacilli and streptococci The topographical distribution of *S albus* and corynebacteria on 12 different anatomical regions of the conjunctiva was examined and showed that both bacteria occurred less frequently on the bulbar regions No differences between the upper and lower parts nor between the outer and inner regions could be found A culture obtained from the lower fornical and tarsal conjunctiva revealed about 93 % of *S albus* and corynebacteria actually harbouring on the conjunctiva
The instillation of anaesthetic eye drops (0 2 % benoxinate hydrochloride) immediately before obtaining cultures was seen to alter the preexisting bacterial distribution on the conjunctiva to preponderance in its lower parts presumably by a washing out effect

Key words conjunctiva – bacteria – topography – topical anaesthetics

Since the appearance of Axenfeld s book *Die Bakteriologie in der Augenheilkunde* in 1907 a milestone in the history of ocular bacteriology the bacterial flora of the normal conjunctiva has been the subject of numerous studies either

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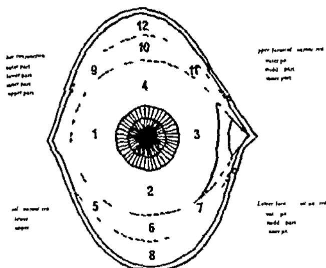


Fig 3
Conjunctival regions examined (drawing reproduced with permission from Kessing S V (1968) Mucous gland system of the conjunctiva Acta ophthal (Kbh) Suppl 95)

Statistical methods The results were analysed at the Department of Bio Statistics Statens Serumsinstitut Copenhagen The comparisons between the number of bacteria found on the different parts of the conjunctiva have been carried out as follows the observations were divided into four groups according to the number of colonies 0 1– 6–25 and > 25 For the pairwise comparisons a difference was registered if the two results fell in different groups the sign of the difference was noted and furthermore a distinction was made between (a) 0 versus > 0 differences and (b) cases where both results were positive The statistical test used was the so called McNemar s test (Armitage 1971)

Results

Incidence and flora

As seen from Tables I and II out of the 100 patients only two showed sterile cultures 45 patients had one kind of bacteria, 48 had two kinds and five had three or more kinds Eighty two patients had *Staphylococcus albus* (coagulase negative) 58 corynebacteria 5 *Staphylococcus aureus* (coagulase positive) 4 proteus 3 streptococci 3 micrococci and 1 *Diplococcus pneumoniae* Table II shows that *S albus* and corynebacteria alone or in combination formed 82 %

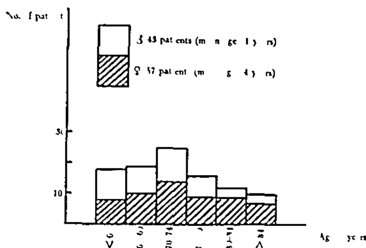


Fig 1
Age and sex distribution of 100 patients examined

had received any antibiotics or cortico steroids during the last weeks and for the sake of homogeneity only senile eyes were chosen As seen from Fig 1 57 patients were females (mean age 74 years) and 43 were males (mean age 71 years) Eighty two patients were older than 65 and only two were younger than 60 years

bacteriological methods By means of a precisely calibrated (0 001 ml = one microliter) platinum loop (Fig 2) samples were taken from 12 different anatomical regions of each conjunctiva (Fig 3) Four cultures were taken from the outer (1) lower (2) inner (3) and upper (4) parts of the *bulbar conjunctiva* Three cultures from the outer (5) middle (6) and inner (7) parts of the *lower fornix* Two from the lower (8) and upper (12) *tarsal conjunctiva* and finally three from the outer (9) middle (10) and inner (11) parts of the *upper fornix*

The samples were immediately inoculated on 5% horse blood agar plates and incubated (37 C) for 48 hours before counting the colonies and identifying bacteria

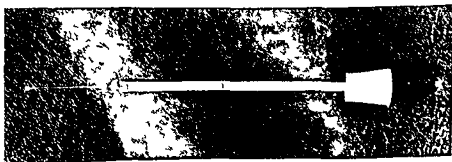


Fig 2
Calibrated platinum loop (0 001 ml = one microliter)

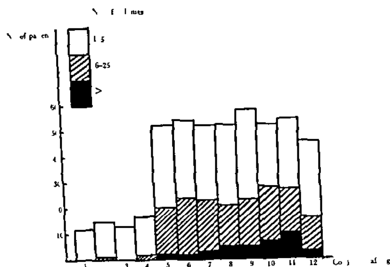


Fig 4

Distribution of *S albus* on 12 different conjunctival regions of 100 patients (*Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part *Lower fornical conjunctiva* (5) outer (6) middle (7) inner part *Tarsal conjunctiva* (8) lower (12) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

of the conjunctival flora There was no significant correlation between the occurrence of both bacteria within the examined regions This held for the incidence as well as for the number of colonies

Topographical distribution

The incidence and density (number of colonies) of *Staphylococcus albus* and corynebacteria on the different parts of the conjunctiva seemed to vary as seen from Figs 4 and 5 *S albus* occurred less frequently on the bulbar conjunctiva than on the other parts whereas for corynebacteria the distribution according to the number of colonies was more equal For both bacteria pairwise comparisons between the 12 locations revealed no differences between the upper and lower parts nor between the outer and inner regions

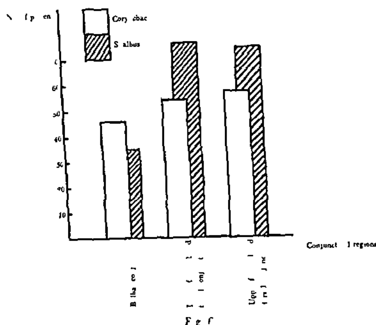
Fig 6 shows the incidence of *S albus* and corynebacteria on three different anatomical regions the *bulbar conjunctiva* (location 1–4 Fig 3) the *lower tarsal and fornical conjunctiva* (5–8) and the *upper tarsal and fornical conjunctiva* (9–12) The incidence of *S albus* was 35 % 76 % and 74 % (total 82 %) and

Table I
Incidence of microorganisms cultured from 100 normal conjunctivas

Microorganisms	Incidence in percent
Staphylococcus albus	82
Corynebacteria	58
Staphylococcus aureus	5
Streptococcus	3
haemolyticus 2	
non haemolyticus 1	
Proteus	4
mirabilis 3	
morgani 1	
Micrococci	3
Diplococcus pneumoniae	1
No growth	2

Table II
Staphylococcus albus and corynebacteria as combined with other microorganisms in 100 normal conjunctivas

Other microorganisms	–*S albus*		+*S albus*		Total
	–coryne bacteria	+coryne bacteria	–coryne bacteria	+coryne bacteria	
None	2	10	32	40	84
S aureus	1	0	1	3	5
Streptococci	0	0	2	1	3
Proteus	1	1	1	1	4
Other gram positive bacteria	1	2	1	0	4
Total	5	13	37	45	100



F g f
Incidence of *S albus* and corynebacteria on 3 different anatomical regions of 100 conjunctivas

the distribution was not homogeneous the variation in the number of colonies between the four locations was much larger than expected if the bacteria were distributed independently and uniformly The differences mentioned above between *S albus* and corynebacteria could be due to the fact that the number of colonies was generally much higher for corynebacteria than for *S albus*

Immediate influence of eye drops on topographical distribution

Out of the 100 patients 5ı were reexamined in the same manner immediately after the application of sterile 0 2 % benoxinate hydrochloride anaesthetic eye drops (with 0 5 % phenylethylalcohol as preservative) The total number of positives among these 5ı patients before the instillation of eye drops was 4ı or 83 % for *S albus* and 31 or 55 % for corynebacteria which was almost the same incidence as for the total group of 100 patients

As seen from Fig ı a tendency to increasing incidence of *S albus* on the bulbar and lower fornical conjunctiva associated with a decrease in the number

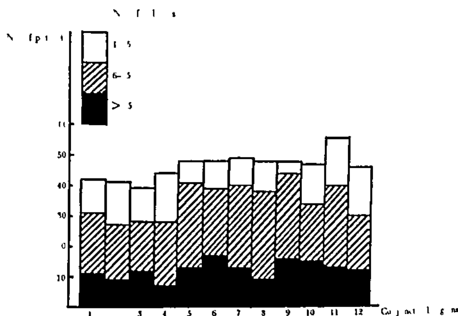


Fig 5

Distribution of *corynebacteria* on 12 different conjunctival regions of 100 patients (*Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part *Lower fornical conjunctiva* (5) outer (6) middle (7) inner part *Tarsal conjunctiva* (8) lower (12) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

of corynebacteria 46 % 54 % and 57 % (total 58 %) respectively Thus for both bacteria about 93 % of the total number of positive cases were observed on the lower tarsal and fornical conjunctiva (5–8) Pairwise comparisons between the three above mentioned regions have been carried out by registering a region as positive if at least one of the four locations was positive For *S albus* the impression from Figs 4 and 6 was confirmed the low incidence in region 1–4 was clearly significantly different from the two other regions (5–8 and 9–12) and no significant difference was found between the latter For corynebacteria it was possible to demonstrate that the incidence was lower on the bulbar conjunctiva (1–4) than in the other two groups ($P = 3.8$ and 0.4 % respectively)

Thus the same tendency was observed for both bacteria but was less pronounced for corynebacteria

The variation in incidence between the locations within each region was greater for *S albus* than for corynebacteria i e the number of cases with all four locations positive was 3 30 and 28 for *S albus* and 34 40 and 38 for corynebacteria or 9 39 and 38 and 74 74 and 67 % of the total number of positives in the different regions For the cases with all four locations positive

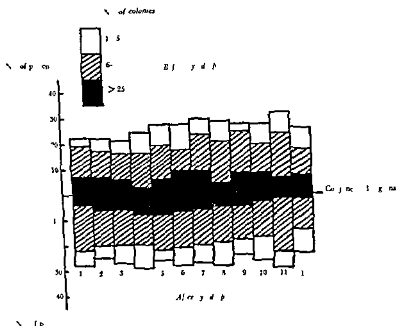


Fig 5
Effect of benoxinate hydrochloride eye drops on the topographical distribution of *cory nebacteria on 12 different conjunctival regions (Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part *Lower fornical conjunctiva* (5) outer (6) middle (7) inner part *Tarsal conjunctiva* (8) lower (12) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

was found Three patients showed bacteria only before the treatment with eye drops The difference between the total number of colonies found before and after treatment showed large variations between the patients but no systematic deviation from zero was observed

Discussion

In agreement with Axenfeld (1907) and many others (Kraupa 1914 Stanka 19 4 McKee 19 4 Edmund 1927 Lucic 1927 Khorazo & Thompson 1935 Kodin 194 Barfoed 1953 Smith 1954 Cason & Winkler 1954 Chang 1957

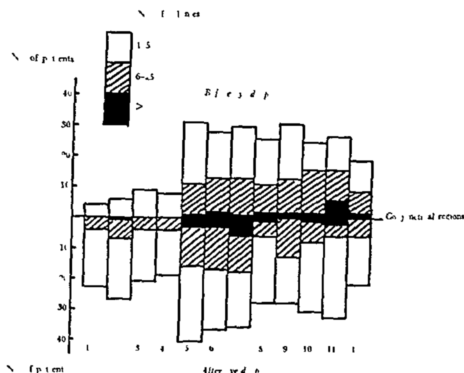


Fig 7

Effect of benoxinate hydrochloride eye drops on the topographical distribution of *S albus* on 12 different conjunctival regions (*Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part *Lower fornical conjunctiva* (5) outer (6) middle, (7) inner part *Tarsal conjunctiva* (8) lower (12) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

of colonies on the upper tarsal and fornical regions was observed as if the bacteria were washed out from the upper to the lower parts The same tendency was also seen in respect to the decrease of the number of colonies of coryne bacteria on the upper areas accompanied by increasing incidence on the bulbar conjunctiva (Fig 8) By the mean of the McNemar s test a significant increase of *S albus* on the bulbar ($P < 0\,0001$) and on the lower fornical conjunctiva ($P < 0\,02$) was proved as well as an increase of corynebacteria on the bulbar conjunctiva ($P < 0\,02$) following the instillation of benoxinate hydrochloride (Table III)

For all three regions together it was found that 44 out of the 57 patients proved to be either positive with the same bacteria or negative before as well as after the application of eye drops Seven cases showed *S albus* and three coryne bacteria only after eyedrops in two of these cases a larger number of colonies

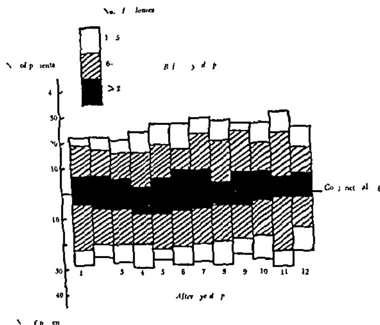


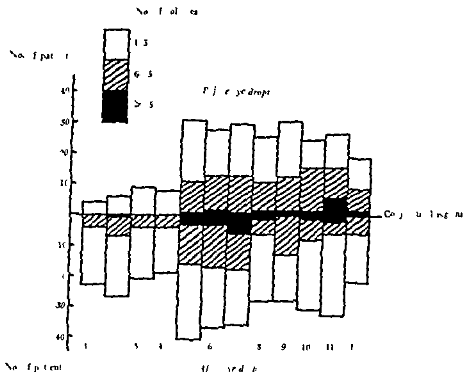
Fig 8

Effect of benoxinate hydrochloride eye drops on the topographical distribution of *corynebacteria* on 12 different conjunctival regions (*Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part. *Lower fornical conjunctiva* (5) outer (6) middle (7) inner part *Tarsal conjunctiva* (8) lower (10) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

was found Three patients showed bacteria only before the treatment with eye drops The difference between the total number of colonies found before and after treatment showed large variations between the patients but no systematic deviation from zero was observed

Discussion

In agreement with Axenfeld (1907) and many others (Kraupa 1914 Stanka 1924 McKee 1924 Edmund 1927 Lucic 1927 Khorazo & Thompson 1935 Kodin 1945 Barfoed 1953 Smith 1954 Cason & Winkler 1954 Chang 1957



Fig

Effect of benoxinate hydrochloride eye drops on the topographical distribution of *S albus* on 12 different conjunctival regions (*Bulbar conjunctiva* (1) outer (2) lower (3) inner (4) upper part *Lower fornical conjunctiva* (5) outer (6) middle, (7) inner part *Tarsal conjunctiva* (8) lower (12) upper part *Upper fornical conjunctiva* (9) outer (10) middle (11) inner part)

of colonies on the upper tarsal and fornical regions was observed as if the bacteria were washed out from the upper to the lower parts The same tendency was also seen in respect to the decrease of the number of colonies of coryne bacteria on the upper areas accompanied by increasing incidence on the bulbar conjunctiva (Fig 8) By the mean of the McNemar s test a significant increase of *S albus* on the bulbar ($P < 0\,0001$) and on the lower fornical conjunctiva ($P < 0\,02$) was proved as well as an increase of corynebacteria on the bulbar conjunctiva ($P < 0\,02$) following the instillation of benoxinate hydrochloride (Table III)

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Doden 1957 Orfila & Courden 1961 de Ocampo et al 1965 Locatcher Khorazo & Seegal 1972) the present investigation demonstrated that *S albus* and corynebacteria are still the most common microorganisms found on the normal conjunctiva and *S aureus* gram negative bacilli and streptococci occurred less frequently However the incidence of the different bacteria especially that of *S aureus* and sterile cultures found in the present and other studies (Fahmy et al 1974 b c) clearly varied from many of the mentioned investigations The probable reasons are discussed elsewhere (Fahmy et al 1974 b c)

The ophthalmic literature is enormous with reports dealing with almost every aspect of the normal ocular flora. However the topographical distribution of bacteria on the normal conjunctiva does not seem to have roused any particular interest Pillat s paper (1921) published half a century ago still remains the only source of our present knowledge Other authors (Eyre 1898 Gowen 193? Locatcher Khorazo & Seegal 1972) claims on the subject seem to be more or less speculative or undocumented

According to Pillat (1921) *S albus* was mainly localised on the upper fornical and tarsal conjunctiva strepto pneumococci on the bulbar while corynebacteria was equally distributed on all parts of the conjunctiva. The present study showed in contradistinction to Pillat that *S albus* and corynebacteria occurred less frequently on the bulbar conjunctiva, while no differences between the upper and lower parts nor between the outer and inner regions could be found No correlation between the occurrance of both bacteria within the regions of the conjunctiva could be proved This held for the incidence as well as for the number of colonies Pillat s (1921) finding that strepto-pneumococci harboured chiefly on the bulbar conjunctiva could not be examined as its incidence (4 %) as well as that of *S aureus* (5 %) in the present study was too low to permit any statistical analysis

One of the interesting results gained from the present study seems to be the fact that about 93 % of *S albus* corynebacteria and probably other organisms actually harbouring on the normal conjunctiva will be revealed by a culture taken from the lower fornical and tarsal conjunctiva as is usually done A sample obtained from the bulbar or tarsal conjunctiva only as is sometimes performed (Lawson 1898 Keilty 1930 Khorazo & Thompson 1935 *Rodin 1947* Olson 1969 Burns 19 ?) would inevitably give a lower incidence of isolations

The instillation of anaesthetic eye drops (0 2 % benoxinate hydrochloride) *into the eye immediately* before obtaining cultures proved to alter the pre existing topographical distribution of *S albus* and corynebacteria on the conjunctiva. A shift was seen from the upper to the lower areas as if the bacteria were washed out from the conjunctiva. Furthermore bacteria primarily not found on the conjunctiva could be recovered after the application of eye drops

Table III

Effects of benoxinate hydrochloride eye drops on the distribution of *S albus* and corynebacteria on 57 normal conjunctivas

Microorganisms	Anatomical regions	Bacterial growth					McNemar s test
		Before eye drops	No growth	Growth	No growth	Growth	
		After eye drops	No growth	No growth	Growth	Growth	Level of significance
S albus	Bulbar con junctiva		16	2	24	15	$P < 0\,0001$
	Lower fornical and tarsal conjunctiva		3	2	11	41	$P < 0\,02$
	Upper fornical and tarsal conjunctiva		10	4	7	36	n s *
Corynebacteria	Bulbar con junctiva		26	1	7	23	$P < 0\,02$
	Lower fornical and tarsal conjunctiva		26	1	2	28	n s *
	Upper fornical and tarsal conjunctiva		25	3	1	28	n s *

* not significant

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Both phenomena are probably due to a reflex outflowing of tears caused by the eye drops both helped by washing off desquamated surface cells upon which bacteria had previously found a suitable culture bed This washing out effect could be clinically used (Fahmy et al 1974 b c) by increasing the isolation rate of bacteria from the conjunctiva

The use of topical anaesthetics before obtaining cultures has been disputed (Erlich 1961 Kleinfeld & Ellis 1966) because of its inhibitory effects on bacterial growth This contention was not confirmed either in the present study or in any other (Fahmy et al 1974 b)

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THE BACTERIAL FLORA OF THE CONJUNCTIVAL ANOPHTHALMIC SOCKET IN GLASS PROSTHESIS CARRIERS

BY

J NYBOE CHRISTENSEN and J A FAHMY

In 44 patients wearing uniocular glass prostheses the bilateral conjunctival bacterial flora was studied and compared The predominant organisms were essentially the same in both sides *Staphylococcus albus* corynebacteria followed by *Staphylococcus aureus* However the incidence of contaminant bacteria (gram negative bacilli and anaerobic bacteria grouped together) was significantly higher ($P < 0.05$) on the socket side
Patients with inflamed sockets proved to harbour more potentially pathogenic bacteria (*S aureus* streptococci and gram negative bacilli) than those without inflammation ($P < 0.05$)
The wearing time of the prosthesis and methods used for keeping it clean did not seem to alter the flora of the socket

Key words bacteria – conjunctival socket – conjunctiva – bacterial flora

Our present concept of the bacterial conjunctival flora is essentially the same as that first stated by Axenfeld (1907) at the beginning of this century *Staphylococcus albus* and corynebacteria constitute the major part of the flora Other organisms such as *Staphylococcus aureus* streptococci and gram negative bacilli may occur but less frequently Under special circumstances the bacterial flora

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Table I

Causes of enucleation of 44 eyes

Causes	No of cases
Blind painful eye	9
Malignant melanoma	9
Injury	6
Corneal diseases	6
Primary glaucoma	5
Vitreous or chorioidal haemorrhages	3
Uveitis	2
Diabetes mellitus	2
Retinal detachment	2
Total	44

Table II

Incidence of the various microorganisms as found in the conjunctival socket and in the fellow conjunctiva of 44 patients

Microorganisms	Orbital socket no of cases	Fellow conjunctiva no of cases
S albus	37	36
Corynebacteria	8	27
S aureus	9	[illegible]
Streptococcus		
haemolytic	0	0
non haemolytic	4	4
Pneumococci	2	0
Gram negative bacteria		
P mirabilis	1	0
E coli	1	0
Klebsiella sp	1	0
B anitratum	2	0
Haemophilus influenzae	1	0
Neisseria sp	0	1
Micrococci	1	1
Anaerobic bacteria		
Clostridium welchii	1	0
Veillonella alcalescens	1	0
No growth	1	3

of the conjunctiva may be altered During acute conjunctivitis *Diplococcus pneumoniae* and *Haemophilus influenzae* dominate while in chronic infections of the conjunctiva *S aureus* was isolated most frequently (Gutierrez 1972) At autopsy Kanski (1965) found increased incidence of gram negative bacilli especially that of *Escherichia coli* The use of contact lenses did not seem to alter the preexisting flora (Winkler & Dixon 1967)

Relatively few authors (Stone 1952 Morgan & Hill 1964 Goldfarb & Turtz 1966) have been interested in the bacterial flora of the conjunctival socket with ocular prostheses The bacteriological results gained from Goldfarb & Turtz s (1966) investigation indicated a higher incidence of streptococci and gram negative bacilli than was usually found in the normal conjunctiva Johnston et al (1966) showed that eye sockets are particularly prone to bacterial contamination and *E coli* is second only to *S aureus* as a socket contaminant The present study was performed primarily to investigate the bacterial flora of the conjunctival socket and compare it with the flora of the fellow conjunctiva to our knowledge such a study has not been made previously and furthermore to correlate the bacterial findings with the signs and symptoms as well as with the hygienic habits of the different patients examined

Material and Methods

The material comprises 44 patients admitted to the Ophthalmological Department of Kommunehospitalet Copenhagen in the period 1 Jan 1970 – 31 Dec 1972 Twenty two patients were females and 22 males the age varied from 7 to 89 years (mean age 67 5 years)

Table I shows the causes of enucleation of the 44 eyes nine were removed as the result of thrombosis of the central vein (blind painful eye) a further nine eyes were enucleated because of ocular malignant melanoma Other causes of enucleation included degenerated eyes caused by injury (six eyes) corneal diseases (six eyes) primary glaucoma (five eyes) vitreous or chorioidal haemorrhages (three eyes) uveitis (two eyes) and retinal detachment (two eyes)

Bacteriological methods

Cultures were obtained from the lower fornical and tarsal conjunctiva with a dry cotton swab after instillation of 0 2 % benoxinate hydrochloride anaesthetic eye drops without preservatives as it was found elsewhere (Fahmy et al 1974b) that the use of anaesthetic eye drops immediately before taking culture increased the isolation rate of bacteria

Table IV

Correlation between the incidence of bacteria found in the conjunctival socket and the fellow conjunctiva

Microorganisms	Growth in A Growth in B	Growth in A No growth in B	No growth in A Growth in B	No growth in A No growth in B	Sign test
S albus	30	5	6	1	***n s
Corynebacteria	19	8	8	8	n.s
S aureus	4	3	5	32	n.s
Streptococci	1	3	3	37	n s
Gram negative bacilli alone	0	0	5	39	n s
Contaminant bacteria	0	0	7	37	$P < 0.05$

A fellow conjunctiva
B conjunctival socket
Gram negative bacilli *P mirabilis E coli* Klebsiella *B antitratum* and *H influen ae*
Contaminant bacteria gram negative bacilli and anaerobic bacteria
n s not significant on 5 % level

Five patients had gram negative bacilli in their sockets (2 *Bacterium anitratum* 1 *Proteus mirabilis* 1 *E coli* 1 *Klebsiella sp* and 1 *H influen ae)* and another two patients had anaerobic bacteria while none were found in the fellow conjunctiva By the means of the sign test (Table IV) the difference was significant ($P < 0.05$) when both were grouped together (as contaminant bacteria)

Correlation with signs and symptoms

Among the 44 patients 26 were found to have inflamed sockets (defined as hyperemia oedema and/or mucopurulent discharge) *while 18* patients had no sign of any inflammation. The incidence of the various bacteria observed alone in both groups was not significantly different (Table V) however *counting* potentially pathogenic bacteria (*S aureus* streptococci and gram negative bacilli) as one group a significant difference could be found Fourteen patients with inflamed sockets showed one or more of the potentially pathogenic bacteria while only three in the group with no inflammation had one or more of those bacteria (Fisher exact test $P < 0.05$) Twenty seven patients complained

Cultures were sent to the laboratory within minutes where they were inoculated on 5 % blood agar plates as well as on serumbouillon and incubated (37 C) for 48 hrs The bacterial identification was undertaken by the usual routine methods however an attempt was further made to culture anaerobic bacteria as well as *H influenzae*

Statistical methods

The *sign test* for paired data (Table IV) and *Fischer exact test* (Tables V VI VII and VIII)

Results

Bacterial flora

As may be seen from Tables II and III the bacterial flora of the conjunctival socket was dominantly the same as that of the fellow conjunctiva *S albus* and corynebacteria were the most common microorganisms followed by *S aureus*

Table III

Bacterial flora of the conjunctival socket and fellow conjunctiva of 44 patients

Microorganisms	Orbital socket no of cases	Fellow conjunctiva no of cases
S albus alone	7	9
Corynebacteria alone	1	1
S aureus alone	1	1
S albus and corynebacteria	16	18
S albus and *S aureus*	1	2
Corynebacteria and *S aureus*	1	1
S albus and corynebacteria and *S aureus*	3	3
Other kinds of bacteria alone or with the first 3 kinds of bacteria	13	5
No growth	1	3
Total	44	43*

* One patient had bilateral eye prostheses therefore only one sample was taken

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S aureus	4	3	5	32	n s
Streptococci	1	3	3	37	n s
Gram negative bacilli alone	0	0	5	39	n s
Contaminant bacteria	0	0	7	37	$P < 0.05$

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Other kinds of bacteria alone or with the first 3 kinds of bacteria	13	5
No growth	1	3
Total	44	43*

* One patient had bilateral eye prostheses therefore only one sample was taken

Table VII

Correlation between bacteria and wearing time of prosthesis in 44 patients

Microorganisms	Wearing time		Total (44 pts)	Fisher exact test Level of significance
	≤ 1 week (32 pts)	> 1 week (12 pts)		
S albus	27	10	37	*n s
Corynebacteria	20	8	28	n s
S aureus	6	3	9	n s
Streptococci	4	2	6	n s
Gram negative bacilli	4	1	5	n s

n s not significant on 5 • level

Correlation with hygenic habits

The material was divided into two groups according to the wearing time of the prosthesis (Table VII) 32 patients wore it for less than a week at a time while 12 patients kept it in for a longer period The incidence of the different bacteria in both groups did not vary significantly

Table VIII

Correlation between bacteria and methods of cleaning of prosthesis in 44 patients

Microorganisms	Methods of cleaning the prosthesis		Total (44 pts)	Fisher exact test Level of significance
	Boric acid or mercury solut (16 pts)	Water or nothing (28 pts)		
S alb	14	23	3	n s
C ryn bacteria		21	28	n s
S au eu	3	6	9	n.s
Streptoc icci	3	3	6	n s
Gram neg ti e bac lli al ne	3	-	5	n s

n s. not significant n 5 level.

Table V

Correlation between bacteria and inflammation of the conjunctival socket

Microorganisms	Inflammation (26 pts)	No inflammation (18 pts)	Total (44 pts)	Fisher exact test Level of significance
S albus	21	16	37	n s*
Corynebacteria	16	12	28	n s
S aureus	7	2	9	n s
Streptococci	6	0	6	n s
Gram negative bacilli^x	4	1	5	n s
Potential pathogenic bacteria^z	14	3	17	$P < 0.05$

^x concerning the individual groups see Table IV

^z this group includes *S aureus* streptococci pneumococci and gram negative bacilli

* n s = not significant on 5 % level

over one or more symptoms (discomfort tearing discharge) whereas 17 had no complaints The incidence of the various bacteria in both groups was not significantly different (Table VI)

Table VI

Correlation between bacteria and subjective symptoms of 44 patients

Microorganisms	Subjective symptoms (27 pts)	No subjective symptoms (17 pts)	Total (44 pts)	Fisher exact test Level of significance
S albus	22	15	37	n s*
Corynebacteria	16	12	28	n s
S aureus	8	1	9	n s
Streptococci	3	3	6	n s
Gram negative bacilli^x	4	1	5	n s

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Microorganisms	Wearing time		Total (44 pts)	Fisher exact test Level of significance
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Corynebacteria	20	8	28	n s
S aureus	6	3	9	n s
Streptococci	4	2	6	n s
Gram negative bacilli	4	1	5	n s

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Correlation between bacteria and methods of cleaning of pros hesis in 44 patients

Microorganisms	Methods of cleaning the prosthesis		Total (44 pts)	Fisher exact test Level of significance
	Boric acid or mercury solut (16 pts)	Water or nothing (28 pts)		
S alb	14	23	37	n s
Corynebacteria	7	21	28	n s
S aureus	3	6	9	n s
Streptococci	3	3	6	n.s
Gram negative bacilli alone	3	2	5	n s

n s not significant on 5 % level.

As to the patient s cleaning habits of the eye region and the prosthesis 13 used mercury solutions or boric acid, while 31 used only water for the prosthesis itself or nothing (Table VIII) Also here no difference between the occurrence of the various bacteria in both groups could be observed

Discussion

According to the results obtained from the present investigation *S albus* and corynebacteria proved to be found just as frequently in the conjunctival socket as in the fellow conjunctiva The same was true for *S aureus* and streptococci

Considering anaerobic microorganisms together with the gram negatives as belonging to the group of contaminant bacteria both were seen more frequently ($P < 0\ 05$) on the socket side This is in agreement with the findings of Goldfarb & Turtz (1966) and Johnston et al (1966)

A decreased lacrimation and frequent wiping could possibly explain the mentioned fact

The wearing time" of prosthesis and the methods used for keeping it clean did not seemed to influence the flora of the socket

It is a well known fact that bacteria with pathogenic properties such as *S aureus* streptococci and gram negative bacilli may be found in eyes without any sign of infection In cases with lowered resistance these bacteria may produce inflammation and discharge as was seen in 14 cases in the present study

The mechanical irritation caused by the prosthesis and the decreased secretion of tears (Norn 1966) with its lysozyme were probably further factors which helped to establish inflammation

Acknowledgement

Miss Elly Norup Sorensen is gratefully acknowledged for performing all the bacteriological laboratory examinations

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CORNEAL TEMPERATURE IN MAN AND RABBIT

Observations made using an infra red camera and a cold chamber

BY

P. RYSÄ and J. SARVARANTA

Using an infra red camera AGA 680 the temperature of the cornea of men and rabbits was measured with a precision of ±0.1°C. The subjects were moved from normal room temperature into a cold chamber with temperatures of 0°C and -10°C.
The material was divided and mean corneal temperatures were recorded at various times as follows

Subject	Cases	Ambient temp (°C)	Body temp (°C)	Corneal temp (°C)		
				15	1	20
Rabbit	21	±0	39.4	35.6	34.6	32.1
Rabbit	57	-10	39.6	35.6	34.[illegible]	31.2
Man	12	-10	37.0	34.8	33.4	31.2

The frequency of blinking is low in rabbit compared to that of man.
On the basis of present data it is obvious that the corneal temperature is a function not only of the ambient temperature but also of body temperature and blinking.

Key words: thermography – temperature of the eye – climate room – arctic eye diseases – anterior chamber depth – cornea – methodology

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It is supposed that there is an additional factor responsible for more rapid cooling in the human cornea than in a rabbit s This factor might be the *smaller amount of aqueous humor behind the cornea in which ase the* corresponding thermal capacity will also be smaller
Using a test animal lacks the advantage of co operation during the test
The physical nature of using the infra red camera in measuring the temperature of the cornea is briefly discussed

Material and Methods

The method has previously been described by Rysa & Sarvaranta 1974 The temperature in the anterior parts of the eye was measured with an infra red camera AGA® model 680 Using a black body heat reference one can determine absolute temperatures with a precision of ±0 1°C (Fig 1) The human *subject taking part in the experiment* sits on a stool and places his head in a



Fig 1
Measuring the corneal temperature in the climate chamber using an *infra red camera* and a black body heat reference

stand so that it remains in a fixed position during the entire measurement period The animal used in the experiment (rabbit) having been put in a special stand is placed in front of the camera to permit measurement

After moving the subject from the room adjacent to the chamber the corneal temperature was recorded at intervals of 15 sec, 30 sec 1 min and 20 min Before each experiment the body temperature of the human subjects was measured with an axillary mercury thermometer and the rectal temperature of the rabbits with a contact thermometer (Lilab® 71 3)

Young healthy adults were used as the subjects for the experiment and healthy albino rabbits as the animals for the experiment The temperature of the centre of the cornea was recorded in 78 rabbit eyes and 12 human eyes The material was divided into three groups according to the temperature in the cold chamber as follows

Group I 21 rabbit eyes temp in the chamber ±0°C
Group II 57 rabbit eyes temp in the chamber −10°C
Group III 12 human eyes temp in the chamber −10°C

The relative humidity in the chamber was kept between 30 and 40 % The mean temperature in the adjacent room was 24 C with a standard deviation of 1 0

Results

The mean values of the temperature measurements together with their standard deviations appear in Table I The results of temperature measurements of the cornea are set out graphically in Figure 2 Slopes and corresponding temperature differences for the measurement periods 15 –1 1 –5 5 –20 and 15 –20 appear in Table II

When the time factor is taken into account it can be established that the greatest changes occur in the period 15 –1 With regard to the slopes it can be stated that for the period 15 –1 curves I and III differ significantly from one another statistically ($t = 2\ 63$ $P < 0\ 02$) The difference between curves I and II over the same period is not statistically significant ($t = 0\ 50$) For the period 15 –20 the slope of curve II shows a statistically significant deviation from the slope of curve I ($t = 1\ 8$ $P < 0\ 10$) and curve III ($t = 3\ 63$ $P < 0\ 001$) For the same period the difference between the slopes of curves I and III is not statistically significant ($t = 0\ 71$)

There is a strong positive correlation between T_t and $T_{t\ 1}$ ($r = +0\ 75$)

Table I

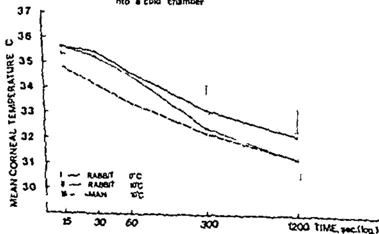
THE MEAN CORNEAL TEMPERATURE AFTER MOVING FROM ROOM TEMPERATURE INTO A COLD CHAMBER

GROUP Nr	OBJECT	CASES	T BODY °C		T out °C		T in °C	CORNEAL TEMPERATURE °C 15		30		1		5		20	
			Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
I	RABBIT	21	39.4	0.5	24.3	0.6	±0	35.6	0.3	35.4	0.4	34.6	0.7	33.1	1.0	32.1	1.1
II	RABBIT	57	39.6	0.3	24.0	1.0	-10	35.6	0.6	35.2	0.6	34.5	0.7	32.4	0.8	31.2	0.8
III	MAN	12	37.0	0.3	23.7	0.5	10	34.8	0.5	34.1	0.7	33.4	0.7	32.2	0.8	31.2	0.7

Table II

THE SLOPE OF CORNEAL TEMPERATURE CHANGE AND CHANGE OF CORNEAL TEMPERATURE (ΔT)

GROUP Nr	OBJECT	CASES	T in °C	T 15 1		1 5		5 20		15 – 20		15 1		1 5		15 20	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
I	RABBIT	21	±0	-7.8	3.2	-3.5	1.3	-0.0m	0.00?	-6.8	1.0	1.0	0.4	-1.4	0.5	-3.4	0.6
II	RABBIT	57	-10	-8.2	3.0	-5.3	1.2	-0.0?	0.00?	-8.7	1.4	-1.1	0.4	2.1	0.5	-4.0	0.7
III	MAN	12	-10	-10.7	2.8	-3.4	0.9	-0.0m	0.00?	-7.1	1.3	-1.4	0.3	-1.4	0.4	-3.6	0.7



Fig

The mean corneal temperature after moving from room temperature into a cold chamber. Graphical presentation.

Discussion

A Method

In thermography surface temperature is measured by means of electro magnetic *radiation emitted from the surface* In rapid thermographic apparatus the detector which is most used recently is the indiumantimonide- (InSb) detector This is a photon detector and thus reacts in relation to the number of quanta unlike thermal detectors which react in relation to the energy absorbed For quantum detectors the Stefan Boltzman law takes the form

$$(1)\ Q = \delta T^3$$

where Q is photon emission photons s^{-1} cm^{-2} and δ is constant (1 52041 10^{11} s^{-1} cm^{-2} K^{-3}) For this reason the rate at which photons are emitted from a black body varies as the third power of the absolute temperature of the object rather than as the fourth power which holds good when examining radiation flux To the formula (1) must be added coefficient ε emissivity if the object is not a black body radiator

Mapstone (1968) has calculated in his bolometric measurements (bolometry is sensitive in the 1–25 μ infra red area) that the emissivity of the cornea is between 0 97 and 1 00 The cornea transmits electromagnetic radiation in the visible part of the spectrum but in the adjacent infra red area (at the level of 1 4 μ) transmission begins to be strongly reduced Above 1 8 μ transmission is only in the order of a few percent until at 2 3 μ transmission no longer occurs (Hartridge & Hill 1917) Thus the cornea can be regarded as a black body radiator when the wavelength is above 2 3 μ The Photovoltage InSb detector is sensitive in the area 0 6–5 6 μ However because of the optics used in the *thermovision camera the minimum level of sensitivity is 2 μ*

Thus deeper tissues of the eye can cause only a small addition in the 2–5 6 μ area to the spectrum emitted by the cornea However although the cornea may completely transmit electro magnetic radiation in the 2–3 μ area this part of the photon spectrum emitted by the black body where the temperature is $\leq$ 310 K represents somewhat less than 1 % in the 2–5 6 μ area Thus the addition caused by the deeper tissues to the emission spectrum of the cornea can be disregarded in practice The pre corneal film formed by the tears is in the order of 7–20 μ (Ehlers 1965) in thickness For water layers of this thickness Plyler & Acquista (1954) have measured the percentage of transmission in the wave length area 2–42 μ According to them anywhere else in the 2–6 μ area than between 3 μ and 6 μ there is a relatively high transmission Because the tears represent water about one third of the emission spectrum of the cornea comes from the pre corneal layer when the InSb detector is used in the 2–5 6 μ area

B Results

On the basis of the results obtained it can be established that the temperature of the cornea is influenced by many factors The corneal temperature drops when the subject is moved into a cold ambient temperature until it reaches a determined final temperature The initial temperature correlates strongly to body temperature *It is interesting to observe that in the same ambient tempera*ture (–10°C) the final corneal temperature reaches the same reading (31 2 C) in rabbits as in humans A human blinks considerably more often than a rabbit. By following *the changes in temperature on the thermo vision camera it can be* readily confirmed that blinking is followed by a rise in corneal temperature For the period 15 –1 the slopes of curves II and III differ significantly from one another statistically There must then be some additional factor responsible for the more rapid cooling in a human cornea than in a rabbit s The greater evaporating surface of a rabbit s cornea lends further support to this supposition One such factor might be the relatively smaller amount of aqueous humor behind the cornea in which case the corresponding thermal capacity will also be smaller

The percentage of the volume of the anterior chamber from the volume of the globe is 14 in a rabbit and 4 in man (Tabulae Biologicae 1947) A rabbit s blinking frequency is low (1–3x/20 min) for which reason blinking can in practice be disregarded when measuring rabbits One great disadvantage however is the lack of co operation in rabbits as compared to humans Measuring corneal temperatures in a cold chamber by means of an infra red camera may have importance when examining the human eye s adaptability to frost and in clarifying the reasons for such common eye diseases in arctic conditions as *pinguecula, pterygium and band shaped keratopathy*

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Thus deeper tissues of the eye can cause only a small addition in the 2–5 6 μ area to the spectrum emitted by the cornea However although the cornea may completely transmit electro magnetic radiation in the 2–5 μ area this part of the photon spectrum emitted by the black body where the temperature is $\leq$ 310 K represents somewhat less than 1 % in the 2–5 6 μ area Thus the addition caused by the deeper tissues to the emission spectrum of the cornea can be disregarded in practice The pre corneal film formed by the tears is in the order of 7–20 μ (Ehlers 1965) in thickness For water layers of this thickness Plyler & Acquista (1954) have measured the percentage of transmission in the wave length area 2–42 μ According to them anywhere else in the 2–6 μ area than between 3 μ and 6 μ there is a relatively high transmission Because the tears represent water about one third of the emission spectrum of the cornea comes from the pre corneal layer when the InSb detector is used in the 2–5 6 μ area

B Results

On the basis of the results obtained it can be established that the temperature of the cornea is influenced by many factors The corneal temperature *drops* when the subject is moved into a *cold ambient temperature* until it reaches a *determined final temperature* The initial temperature correlates strongly to body temperature It is interesting to observe that in the same ambient temperature (–10 C) the final corneal temperature reaches the same reading (31 2°C) in rabbits as in humans A human blinks considerably more often than a rabbit. By following the changes in temperature on the *thermo vision camera it can be readily confirmed* that blinking is followed by a rise in corneal temperature For the period 15 –4 the slopes of curves II and III differ significantly from one another statistically There must then be some additional factor responsible for the more rapid cooling in a human cornea than in a rabbit s The greater evaporating surface of a rabbit s cornea lends further support to this supposition One such factor might be *the relatively smaller amount of aqueous humor* behind the cornea in which case the corresponding thermal capacity will also be smaller

The percentage of the volume of the anterior chamber from the volume of the globe is 14 in a rabbit and 4 in man (Tabulae Biologicae 1947) A rabbit s *blinking frequency is low (1–3x/20 min) for which reason* blinking can in practice be disregarded when measuring rabbits One great disadvantage however is the lack of co operation in rabbits as compared to humans Measuring corneal temperatures in a cold chamber by means of an infra red camera may have importance when examining the human eye s adaptability to frost and in *clarifying the reasons for such* common eye diseases in arctic conditions as pinguecula pterygium and band shaped keratopathy

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TONOGRAPHY AND GLAUCOMATOUS OPTIC NERVE DAMAGE

BY

PEKKA E J POHJANPELTO

The diurnal curves and tonography result were studied in 3(7 eyes of 236 patients with ocular hypertension ≧ 20 mmHg The outflow values of 97 eyes with glaucomatous optic nerve damage (OND) were compared with those of eyes with similar pressure but without optic nerve damage. The coefficients were lower in the former than in the latter group but the differences were small and deviations great Tonography performed in addition to the diurnal curve seems to be of very limited value in predicting the risk of glaucomatous damage in an individual eye with ocular hypertension
The outflow coefficient was lower for eyes with the pseudoexfoliation syndrome than for eyes with similar pressure without pseudoexfoliation

Key words tonography – aqueous humor dynamics – ocular hypertension – glaucoma – pseudoexfoliation – optic nerve

The pressure tolerance of individual eyes differs greatly (Pohjanpelto & Palva 19(4) It is therefore difficult to decide the need for therapy for an eye with elevated pressure but no glaucomatous optic nerve damage (OND) Effective *prophylaxis* can lead to the unnecessary treatment of a very great number of patients

Patients with chronic simple glaucoma have repeatedly been shown to have a marked reduction in outflow facility (Duke Elder 1969) In the available literature tonography of glaucomatous eyes has been compared with the results

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obtained for eyes in which the pressure is lower As a lowered coefficient of outflow is correlated with elevated intraocular pressure (Fisher et al 1960) it seemed appropriate to compare outflow in eyes with glaucomatous damage with outflow in undamaged eyes with similar pressure The purpose of the study was to establish whether the addition of tonography to the diurnal curve provides any supplementary information of value in assessing the risk of glaucomatous damage in an eye without OND

Material and Methods

The study was based on the tonography results and diurnal curves of patients with ocular tension ≥ 20 mmHg and open chamber angles who were admitted to the Eye Department Regional Hospital of Lahti for examination in 1969 1972 Some patients with OND who were examined in 1973 are also included At the pressure level of 25–29 mmHg patients without OND and pseudoexfoliation (P) were so numerous that only 80 eyes of a randomly selected group of 52 patients were included

Pressure measurements of an individual patient before the institution of possible therapy generally numbered 6–10 The highest pressure recorded repeatedly was taken as the pressure value for an individual eye The tonography result (Mueller & Co) was read from the tables for average ocular rigidity (Becker & Shaffer 1965) Curves that were a failure technically were discarded

The OND+ group contained the eyes assessed to have excavated disk and a visual field defect and in the OND– group were the eyes with no visual field defect and a disk assessed as normal

The series comprised 367 eyes of 236 patients Ninety seven eyes had OND The pseudoexfoliation syndrome was seen in 112 eyes of 87 patients

The material and methods have been reviewed in greater detail in an earlier work (Pohjanpelto & Palva 1974)

Results

The average coefficients of outflow facility at different pressure levels are given in Table I As the pseudoexfoliation syndrome may impair outflow (Pohjanpelto 1973) the P+ eyes were treated separately in the analysis of the results

Table I

Outflow values (C) in different grades of ocular hypertension

Intraocular pressure (mmHg)	P–						P+					
	OND+			OND-			OND+			OND-		
	No of eyes	C value		No of eyes	C value		No of eyes	C value		No of eyes	C value	
		Mean	Range		Mean	Range		Mean	Range		Mean	Range
20–24	24	0 17	0 08–0 34	57	0 23	0 08–0 52	2	0 13	0 12–0 13	13	0 19	0 11–0 27
25–29	12	0 21	0 11–0 49	80	0 24	0 06–0 59	3	0 15	0 12–0 20	27	0 18	0 04–0 35
30–34	6	0 18	0 09–0 31	54	0 20	0 03–0 57	5	0 11	0 09–0 13	16	0 14	0 05–0 19
35–39	3	0 10	0 08–0 12	9	0 13	0 06–0 23	7	0 11	0 05–0 23	5	0 12	0 03–0 18
40 <	8	0 09	0 00–0 15	2	0 13	0 11–0 14	27	0 07	0 09–0 15	7	0 10	0 05–0 17

OND = Glaucomatous optic nerve damage P = Pseudoexfoliation syndrome

Coefficients of outflow fell when intraocular pressure rose as expected The means were lower at all pressure levels in the OND+ than in the corresponding OND– eyes and the difference was statistically significant ($P < 0.01$) The deviations were considerable however and the differences small If the P+ and P– eyes are taken separately there was no significant difference between the OND+ and OND– eyes in the former group but in the latter group the difference was significant ($P < 0.01$)

Coefficients of outflow were lower in P+ eyes than in P– eyes with the same intraocular pressure ($P < 0.01$)

Table II presents the eyes according to the pressure reading at tonography The aim was to establish whether the coefficient of outflow and the highest value of the diurnal curve are correlated Eyes with a high pressure over 34 mmHg were omitted as the tonography result was almost always so low that it was not worth dividing the eyes into groups When the coefficient of outflow was low the mean of the maximum pressure values was slightly higher than in eyes with a similar pressure and good outflow The difference was statistically significant ($P < 0.01$)

Table II

Tonography results and the maximum pressure of the diurnal curve

Po	C	No of eyes	Maximum pressure	
			Mean	S D
< 20	< 0.13	8	28.3	4.0
	0.13–0.17	10	27.7	3.8
	0.17 <	59	27.8	3.3
20–24	< 0.13	0	29.6	4.7
	0.13–0.17	27	27.9	3.4
	0.17 <	60	28.1	3.3
25–29	< 0.13	16	34.4	8.1
	0.13–0.17	15	31.0	3.4
	0.17 <	38	30.[illegible]	4.0
30–34	< 0.13	19	40.3	8.2
	0.13–0.17	5	35.4	5.3
	0.17 <	17	33.6	4.2

Discussion

The coefficient of outflow was lower in the eyes with glaucomatous damage than in undamaged eyes with the same pressure in this series However the differences between the OND+ and OND– eyes were small and the deviations great. It thus seems that tonography performed in addition to the diurnal curve is only of limited value in assessing the therapeutic requirement of an individual eye

It has been stated that eyes with impaired facility of outflow are more prone to greater pressure fluctuations when the secretory rate varies (Podos & Becker 1973) In the present study the difference between eyes with good and low outflow was surprisingly small when the maximum values of the diurnal curves for eyes with a similar pressure at the time of tonography were compared However it must be remembered that conditions were stable while the patients were in hospital and the number of pressure measurements was limited The pressure variation is possibly greater in ordinary conditions and the difference between eyes with low and good outflow may be greater than was observed in this work.

This may explain why OND+ eyes had a lower outflow than OND– eyes. Although the pressures were the same at the time of examination higher pressure peaks may be encountered in the eye with poor outflow at other times.

The coefficient of outflow was lower in P+ eyes than in P– eyes with the same pressure level This and the aforesaid may be why the incidence of glaucomatous damage is higher in P+ than in P– eyes with the same pressure (Pohjanpelto & Palva 1974)

The outflow coefficient is no separate criterion additional to elevated [illegible] when the risk of glaucoma is evaluated It is an indication of the [illegible] disturbed dynamics of the aqueous humour Its connection with [illegible] more remote than that of ocular tension Outflow facility [illegible] and if this exceeds the tolerance of the eye the result is glaucoma [illegible] to institute therapy on an OND– eye must be based on [illegible] intraocular pressure The significance of tonography [illegible] information in this respect is small However poor [illegible] motivate a closer observation of the patient, and [illegible] low coefficient of outflow speaks in favour of inst[illegible]

Refe[illegible]

Becker B & Shaffer R N (1965) *Dia[illegible]* [illegible] nd ed. Mosby St Louis

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Coefficients of outflow were lower in P+ eyes than in P– eyes with the same intraocular pressure ($P < 0.01$)

Table II presents the eyes according to the pressure reading at tonography The aim was to establish whether the coefficient of outflow and the highest value of the diurnal curve are correlated Eyes with a high pressure over 34 mmHg were omitted as the tonography result was almost always so low that it was not worth dividing the eyes into groups When the coefficient of outflow was low the mean of the maximum pressure values was slightly higher than in eyes with a similar pressure and good outflow The difference was statistically significant ($P < 0.01$)

Table II

Tonography results and the maximum pressure of the diurnal curve

Po	C	No of eyes	Maximum pressure	
			Mean	S D
< 20	< 0.13	3	25.3	4.0
	0.13–0.17	13	25.7	5.8
	0.17 <	31	27.3	3.3
20–24	< 0.13	20	26.6	4.7
	0.13–0.17	27	27.3	3.4
	0.17 <	60	23.1	3.3
25–29	< 0.13	16	34.4	3.1
	0.13–0.17	15	31.0	3.4
	0.17 <	35	30.2	4.0
30–34	< 0.13	19	40.3	3.2
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The outflow coefficient is no separate criterion additional to elevated pressure when the risk of glaucoma is evaluated It is an indication of the same thing disturbed dynamics of the aqueous humour Its connection with glaucoma is more remote than that of ocular tension Outflow facility affects the pressure and if this exceeds the tolerance of the eye the result is glaucoma The decision to institute therapy on an OND− eye must be based on established elevation of intraocular pressure The significance of tonography as a source of additional information in this respect is small However poor facility of outflow may motivate a closer observation of the patient and in a borderline case a low coefficient of outflow speaks in favour of instituting therapy

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	0.17 <	39	[illegible]	3.3
20–24	< 0.13	20	29.6	4
	0.13–0.17	27	[illegible]	3.4
	0.17 <	60	25.1	3.5
25–29	< 0.13	16	34.4	3.1
	0.13–0.17	15	31.0	3.4
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References

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both an activator of fibrinolysis (Pandolfi 1967) and a protein antigenetically related to the von Willebrand factor Factor VIII (vW AHF) (Pandolfi et al 1974). Von Willebrand factor is necessary for platelet adhesion and aggregation (Bouma et al 1972, Holmberg & Nilsson 1973).

The present paper deals with the biological and immunological assay of vW AHF in the blood of diabetic patients with and without ophthalmoscopically visible diabetic retinopathy.

Material and Method

The clinical material consisted of 68 randomly selected patients with DM. They were classified according to the sex, age group (in decades), body weight (10 % under, between ± 10 % and 11–20 %, 21–30 %, > 30 % over the ideal weight), the duration of the disease (up to 3 months, 5, 10, 20, > 20 years after the diagnosis) and the treatment (diet alone, insulin, sulphonylurea, biguanide).

AHF determination. The AHF (antihaemophilia A) activity of the patient plasma was assessed from its normalising effect on the recalcification time of platelet rich haemophilia A plasma containing less than 1 % AHF than normal. The amount of AHF was expressed as percentage of that found for a normal standard consisting of pooled plasma from 10 healthy persons (Nilsson et al 1971).

Determination of vW-AHF related protein. The method is described in detail elsewhere (Holmberg & Nilsson 1973). Briefly, a monospecific precipitating anti AHF antiserum was raised by injecting rabbits with a purified AHF preparation obtained by filtering plasma fractions I-O on a column with Sepharose 6B and using an eluting buffer containing dextran 40. The AHF related protein was quantitatively determined by electrophoresis in agarose gel containing antibodies by the *rocket* technique of Laurell (1966). The electrophoresis is shown in Fig. 1.

Other determinations. They included fibrinogen, cholesterol, triglycerides, alpha₂ macroglobulins, blood and urinary glucose.

Results

According to the ophthalmoscopy the patients fell into three groups:

1. Without diabetic retinopathy (35)

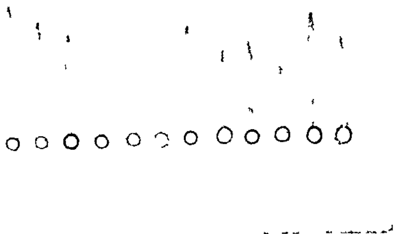


Fig 1
Electrophoresis of von Willebrand AHF related protein in an antibody containing agar gel The height of the peaks is directly related to the amount of antigen.

2 With retinopathy (28) Six had minimal changes consisting of scattered aneurysms The other 22 had retinal bleedings and exudates of various degrees of severity None had vitreous haemorrhages

3 With complications (glaucoma cataract) making a firm diagnosis of retinopathy impossible (5) These patients were excluded

Both the vW AHF related protein and the AHF activity were increased in the blood of diabetics (Table I) The vW AHF related protein was significantly ($0.02 > P > 0.01$) higher in the patients with retinopathy than in those without retinopathy while the AHF activity of both groups was comparable (Table I) The level of vW AHF related protein was not related to the duration of the disease the protein being highest at an intermediate disease duration (Fig 2)

Additional statistical analysis failed to show any correlation between the vW AHF related protein and AHF activity on the one hand and sex age body weight type of treatment blood and urinary glucose, blood fibrinogen cholesterol triglycerides and alpha macroglobulins on the other

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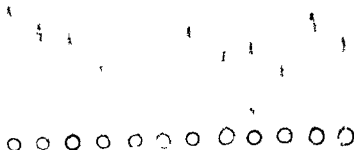


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Table 1

Von Willebrand AHF related protein and AHF activity in the blood of diabetic patients with and without retinopathy Means ± S E and ranges expressed in % of normal values Within bracket the number of patients

	Von Willebrand AHF related protein	AHF activity
Whole material	*160 1 ± 11 56* 530–59 (53)	*151 7 ± 9 39* 500–62 (57)
Patients with retinopathy	195 9 ± 27 9 530–57 (19)	157 5 ± 13 1 315–62 (25)
Patients without retinopathy	140 0 ± 7 45 225–59 (34)	147 2 ± 13 4 500–72 (3[illegible])

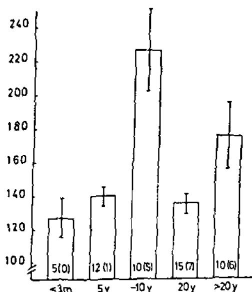


Fig [illegible]

Variations of von Willebrand AHF related protein with the duration of diabetes On the abscissa the duration of diabetes in months and years On the ordinate the blood level of the protein expressed in % of the normal value Vertical bars denote S E Inside the columns the number of patients (patients with retinopathy within brackets)

Comments

In diabetes mellitus and especially in diabetes with vascular complications such as retinopathy increased platelet adhesion and aggregation have been observed (Badawi et al 1970 Heath et al 1971 Kwaan et al 1972 Regnault 1972 Shaw et al 1967 Szirtes 1970) It has been proposed that these changes play a role in the pathogenesis of microangiopathy Regnault (1972) suggests that the first step in the development of retinopathy is an occlusion of small retinal arteries and capillaries by aggregates of platelets which is followed by vascular congestion in the adjoining areas of retina

The von Willebrand factor is a plasma protein which has been found necessary for platelet adhesion (Bouma et al 1972 Cronberg & Holmberg 1973 Salzman 1963) According to recent findings (Bouma et al 1972 Holmberg & Nilsson 1972) this factor is a protein normally having AHF activity which can be detected in the blood by immunological techniques The present results show that the von Willebrand factor is increased in diabetics particularly in those with retinopathy The high level of this factor may play a role in the increased platelet adhesion and aggregation in diabetics and thus be of significance in the development of retinopathy Consistent with this hypothesis is the recent observation that the von Willebrand factor is selectively confined to the endothelium of the retinal vessels (Pandolfi et al 1974) i e in a position where it can readily interact with blood platelets

The impairment of the fibrinolytic mechanism in diabetics which is especially marked in patients with retinopathy (Almer et al 1974) may be expected to lead to an incomplete dissolution of the fibrin at the sites of the platelet aggregates and consequently to aggravate the obstruction of the retinal vessels

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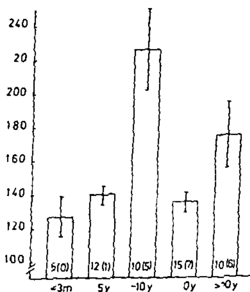


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The Department of Physiology (Head: Professor A. Bill),
the Department of Pharmacology (Head: Professor E. H. Bárány)
and the University Eye Clinic (Head: Professor G. von Bahr),
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EFFECTS OF ARTIFICIAL INTRAOCULAR PRESSURE ELEVATION ON THE OUTFLOW FACILITY AND THE ULTRASTRUCTURE OF THE CHAMBER ANGLE IN THE VERVET MONKEY (Cercopithecus ethiops)

BY

BJÖRN SVEDBERGH

Both eyes of anaesthetized vervet monkeys were perfused with mock aqueous humor for 3–7 hours. By adjusting the height of a reservoir connected to each eye the intraocular pressure in one eye was maintained at 33–48 mmHg and in the other eye it was a few mmHg above the spontaneous level. The facility of outflow in the high pressure eye increased by an average of 350 % (160–630 %) whereas the increase in the control eye was less than 40 %. Morphologically the control eyes appeared normal whereas pronounced changes were observed in the high pressure eyes. The *endothelial cells in the trabecular meshwork were swollen* and demonstrated loss of cytoplasm. Cell debris and *blebs* were observed in the intertrabecular spaces. The endothelial meshwork and the endothelium of the inner wall of Schlemm's canal were partly disrupted. The outflow facility was normalized within 1 day but marked morphological changes were still present.

Key words: Schlemm's canal – trabecular meshwork – scanning electron microscopy – transmission electron microscopy – aqueous outflow facility – glaucoma – intraocular pressure

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EFFECTS OF ARTIFICIAL INTRAOCULAR PRESSURE ELEVATION ON THE OUTFLOW FACILITY AND THE ULTRASTRUCTURE OF THE CHAMBER ANGLE IN THE VERVET MONKEY (Cercopithecus ethiops)

BY

BJÖRN SVEDBERGH

Both eyes of anaesthetized vervet monkeys were perfused with mock aqueous humor for 3–4 hours. By adjusting the height of a reservoir connected to each eye the intraocular pressure in one eye was maintained at 33–48 mmHg and in the other eye it was a few mmHg above the spontaneous level. The facility of outflow in the high pressure eye increased by an average of 350 % (160–630 %), whereas the increase in the control eye was less than 40 %. Morphologically the control eyes appeared normal whereas pronounced changes were observed in the high pressure eyes. The endothelial cells in the trabecular meshwork were swollen and demonstrated loss of cytoplasm. Cell debris and *blebs* were observed in the intertrabecular spaces. The endothelial meshwork and the endothelium of the inner wall of Schlemm's canal were partly disrupted. The outflow facility was normalized within 1 day but marked morphological changes were still present.

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Approximately 75 % of the total resistance to aqueous humor outflow is located in the tissue between the anterior chamber and the *canal of Schlemm* in enucleated normal human eyes (Grant 1958 Moses 1971) and monkey eyes (Peterson & Jocson 1974) Much work has been done to clarify the morphology of this region in glaucomatous eyes Until recently it has been mostly eyes with advanced stages of glaucoma simplex that could be studied The trabeculectomy operation has now made possible the study of early glaucomatous changes in the excised specimen though often associated with traumatic artefacts In such material Rohen & Witmer (1972) observed homogenous osmiophilic plaques in the trabeculum cribriforme (pore tissue juxtacanalicular connective tissue endothelial meshwork) as well as extreme hyalinization of the trabecular lamellae Tripathi (1972) also described hyalinization of the trabeculae but the most *significant finding* was a quantitative and qualitative depletion of the giant *vacuoles in the* inner wall endothelium of *Schlemm s canal* These vacuoles are in fact invaginations from the *trabecular side* some serving as drainage routes for the aqueous humor (Holmberg 1965 Inomata et al 1972) Fink et al (1972) on the other hand could find no significant difference from normal eyes

The morphological effects of artificial changes in the intraocular pressure (IOP) have been studied in several laboratories during the past few years Johnstone & Grant (1973) in a study on the light microscopic level described widening of the trabecular spaces and a narrowing and partial obliteration of *Schlemm s canal* after a period with high eye pressure But with an intraocular pressure of zero Schlemm s canal was distended the gaint vacuoles were non existent and the trabecular meshwork was compressed The findings were similar in experiments *in vivo* on rhesus monkeys and *in vitro* on enucleated human eyes Grierson & Lee (1973) and Grierson (1974) using light and electron microscopy on the *rhesus monkey* found a narrowing of Schlemm s canal and a *corresponding* increase in the thickness of the endothelial meshwork at an elevated IOP The giant vacuoles were prominent and a significant increase in their number was demonstrated The periods with high eye pressure in the experiments mentioned were rather short and the outflow facilities were not measured

The purpose of the present study was *to examine* the effect of a moderate rise *in eye pressure* lasting some hours on the outflow facility and the ultrastructure of the chamber angle tissue Observations of the corneal endothelium in the same eyes are presented elsewhere (Svedbergh 1974c) Preliminary reports of the results in this study were presented at the XXI Meeting of Nordic Ophthalmologists at Åbo Finland June 1973 (Svedbergh 1974a) and at the Symposium *The Application of Electron Microscopy to Ophthalmic Anatomy and Pathology* at Glasgow Scotland September 1973 (Svedbergh 1974b)

Material and Methods

Thirteen adult vervet monkeys (*Cercopithecus ethiops*) of both sexes and weighing 2 5–3 65 kg were investigated No signs of disease in the anterior segment were observed by slit lamp examination The effect of longstanding elevation of the IOP on the facility of outflow was determined in eight monkeys The effect on the structure of the chamber angle was examined in nine animals A complete experiment *will now be* described

Experimental procedure

The experimental setup is shown schematically in Fig 1 Anaesthesia was induced with sodium methohexital (Brietal Sodium® Lilly) about 70 mg/kg body weight given i m Anaesthesia was maintained with pentobarbital sodium given in small doses every 20–30 min by means of a cannulated tail vein The animal was kept warm under a heating pad (rectal temperature 37–38 C) and placed prone with the head in horizontal position in a headholder Three cannulae previously connected to their respective polyethylene tubing were shot into the lower temporal part of the anterior chamber with a needle gun (Sears 1960) and were then turned so that the bevels did not face each other or the cornea The eyelids could cover most of the cornea after the cannulation was performed One cannula connected the eye to a pressure transducer (EMT 31 Elema Schonander Solna Sweden) for measuring the IOP Another cannula connected it to an external *reservoir* the height of which could be regulated and the weight of which was determin

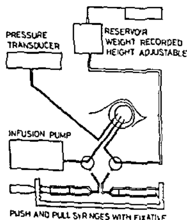


Fig 1

Schematic presentation of the experimental setup Each eye was connected by three cannulae to a pressure transducer a reservoir and an infusion pump After the period of high eye pressure the reservoir and the infusion pump *were disconnected* and the push pull coupled syringes connected for fixation. For details see text

Approximately 75 % of the total resistance to aqueous humor outflow is located in the tissue between the anterior chamber and the canal of Schlemm in enucleated normal human eyes (Grant 1958 Moses 1971) and monkey eyes (Peterson & Jocson 1974) Much work has been done to clarify the morphology of this region in glaucomatous eyes Until recently it has been mostly eyes with advanced stages of glaucoma simplex that could be studied The trabeculectomy operation has now made possible the study of early glaucomatous changes in the excised specimen though often associated with traumatic artefacts In such material Rohen & Witmer (1972) observed homogenous osmiophilic plaques in the trabeculum cribriforme (pore tissue juxtacanalicular connective tissue endothelial meshwork) as well as extreme hyalinization of the trabecular lamellae Tripathi (1972) also described hyalinization of the trabeculae but the most significant finding was a quantitative and qualitative depletion of the giant vacuoles in the inner wall endothelium of Schlemm s canal These vacuoles are in fact invaginations from the trabecular side some serving as drainage routes for the aqueous humor (Holmberg 1965 Inomata et al 1972) Fink et al (1972) on the other hand could find no significant difference from normal eyes

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mine and studied by light microscopy Ultrathin sections were prepared on LKB Ultra tome II and III and Reichert OmU3 and doublestained with a saturated solution of uranyl acetate and lead citrate (Reynolds 1963) The microscope used for SEM were a Stereoscan (Cambridge Instrument Company England) and a JSM U3 (Japan Electron Optics Laboratory Tokyo Japan) and for TEM an EMU 3B (Radio Corporation of America U S A) and a JEM 100B (Japan Electron Optic Laboratory Tokyo Japan)

Results

During the period with high eye pressure a moderate dilatation of the pupil occurred whereas no distinct corneal haze could be observed by slit lamp examination except adjacent to the needle holes When the IOP was lowered to the spontaneous level in the four eyes subjected to facility studies alone a reactive hyperaemia in the iris was observed after 5–10 min in one eye even a hyphaema

Facility of outflow

The gross facilities determined at the start of the experiments range from 0 34–1 1 µl min⁻¹ mmHg⁻¹ During the period with high IOP the facility increased by 160–630 % with a mean value of about 350 % in eight animals (Fig 2) The maximum facility values were reached at 2–3 hours Parenthetically after the period with high IOP attempts were made to determine facility in the initial way from the initial spontaneous IOP in four eyes However due to leakage of blood into the anterior chamber and possibly other factors the determined facility values were 20–90 % lower than the final values determined during the period with high IOP Or considered from another aspect the determined facility values were 50–300 % of the facility values at the start of the experiment Moreover ideal steady state conditions were often hard to obtain Thus these facility determinations were considered of doubtful value and omitted

The facility also changed in the control eyes At the end of the experiment it was 90–140 % of the starting value in the four eyes determined The facility determinations 1 3 and 10 days after a period with high eye pressure resulted in values differing less than 20 % from the initial values in the high pressure eyes as well as in the control eyes

Morphology

The dissection of the inner wall of Schlemm s canal was more difficult than in a previous study on human eyes (Bill & Svedbergh 1972) due to a greater num

ed by a weight sensing transducer (SG4–1 Swema Stockholm Sweden) according to Barany (1966) The last cannula connected the eye to a syringe driven by an electrical motor device The perfusion fluid was mock aqueous humor (Barany 1964) sterilized by passing it through a Millipore filter (Swinnex 13 Millipore Corp Bedford Mass U S A) before using Both eye pressures and the weights of the reservoirs were continuously recorded on a multichannel strip chart recorder (PR 7/91 00 Philips)

After shooting the three cannulae into the anterior chamber of each eye the IOP was allowed to stabilize for 5–10 min with the reservoirs and infusion pumps disconnected by clamps The reservoirs were then connected and adjusted in height to give an IOP 3–4 mmHg above the spontaneous level The inflow from the reservoirs was determined over a 7–10 min period The reservoirs were then elevated to increase the IOP another 7–8 mmHg and the inflow was determined over a 7–10 min period The gross facility of outflow was calculated as the ratio of increase in flow ($\triangle$ Q) to increase in pressure ($\triangle$ P) i e $\triangle Q/\triangle P$ After this initial facility determination the infusion pumps were started with an infusion rate of about 20 µl/min (identical for both eyes) and the reservoirs were adjusted to give an IOP of 33–48 mmHg in one eye (high pressure eye) and 2–3 mmHg above the spontaneous level in the other eye (control eye) During the period with high eye pressure the rate of infusion from the syringes was identically adjusted in such a way that there was a moderate outflow from the reservoir connected to the high pressure eye The period with high eye pressure lasted for 3–7 hours Meanwhile changes in the gross facility were determined every 10–15 min from the ratio between the net inflow (from the reservoir and the infusion pump) and the increase in the IOP above the initial spontaneous level The mean value of the last three determinations was considered as the final value

After the period with high eye pressure the IOP was lowered to the initial spontaneous level The reservoirs and infusion pumps were then disconnected and a pair of push pull coupled syringes (Bill & Barany 1966) containing fixative (Millipore filtered 4 % cold glutaraldehyde) were connected to each eye (Fig 1) The animal was killed by opening the heart and simultaneously fixation was started by perfusion from the push pull coupled syringes This perfusion started 1–2 min after lowering of the IOP and lasted 10–15 min care being taken to maintain the IOP at the initial spontaneous level In three animals the eyes were not fixed immediately after the period with high eye pressure but after 1 3 and 10 days respectively The gross facility was determined before the fixation

Histological procedure

The subsequent steps of dissecting and processing for SEM have been described in detail earlier (Bill & Svedbergh 1972) In short it consisted in further fixation in glutaraldehyde after dissection of the anterior segment and postfixation in osmium tetroxide followed by different dehydration and drying procedures (air drying by ether or ethanol freeze drying by amyl acetate or 100 % ethanol (Boyde et al 1972)) The tissue specimens were then placed on adhesive tape glued on standard stubs and coated with carbon and gold or gold palladium by evaporation in vacuum For TEM the steps of procedure were identical to those for SEM up to 70 % ethanol At this stage the final dissection into less than 2 × 3 × 3 mm pieces was made before passing the specimens for 30 min each in 95 and 100 % ethanol The embedding was done in Epon 812 via propylene oxide Sections 1 µm thin were stained with toluidine blue and paraphenylenedia

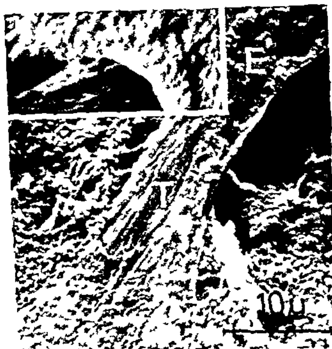


Fig 3
U eal meshwork The covering endothelial layer (E) is lost in places leaving the under lying trabecula bare (T) *Insert corresponding area from a control eye The preparations were air dried with ethanol*

cell debris that was observed in the intertrabecular spaces by TEM In the cytoplasm cell organelles such as the mitochondria the endoplasmic reticulum and the Golgi apparatus were disintegrated and vacuolization was frequently observed The nuclei of the endothelial cells showed peripheral condensation of the heterochromatin with a clear perinuclear zone about 200 Å wide which was only interrupted at the sites of the nuclear pores (Fig 5) Throughout the intertrabecular spaces one could observe many blebs (Figs 4 5) looking like small balloons and measuring 0 5–5 μm They had a thin membrane about as thick as the plasma membrane and were electron optically empty or contained granular material or small vesicles

In the endothelial meshwork the extracellular fibrillar and ground substance had vanished at many sites The cells showed slight oedema and an occasional loss of cytoplasm but had in general a much more electron dense structure in

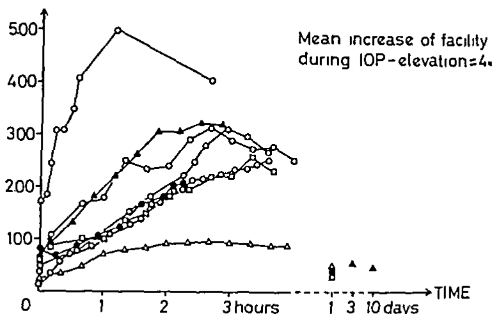


Fig 2
Facility of outflow before during and after the period of high eye pressure in 8 eyes The average increase of facility during IOP elevation is approximately 350 % meaning a reduction of resistance to outflow by approximately 75 % The facility has returned to normal level in 1 day Symbols △ ● □ ▲ denote the same eye

ber of septa bridging over the lumen of the canal The different drying procedures applied for SEM investigation gave no appreciable differences in the results as judged from the control eyes the appearance of which were in accordance with the earlier studies on normal morphology (Bill 1970 Bill & Svedbergh 1972)

The trabecular meshwork

The trabecular cores had a basically normal structure with the exception of occasional electron optically empty spaces presumably representing fluid uptake as these spaces were most often observed where the endothelial lining was severely disintegrated or missing The endothelial cells were diffusely oedematous with an uneven cell surface and the plasma membrane was disrupted in many places (Figs 3 4 5) The unevenness of the surfaces was conspicuous when observed by SEM this was probably due to precipitation of the free floating

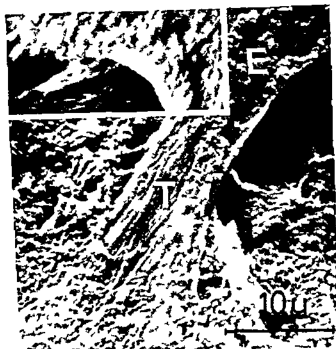


Fig 3

Uveal meshwork The covering endothelial layer (E) is lost in places leaving the under lying trabecula bare (T) Insert corresponding area from a control eye The preparations were air dried with ethanol

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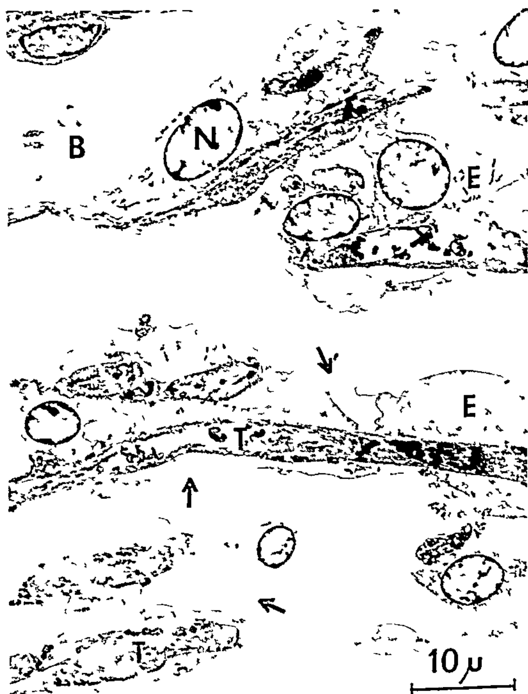


Fig 4
Corneoscleral meshwork The endothelial cells (E) are swollen the nuclear chromatin condensed in the periphery of the nucleus (N) The cytoplasm is disrupted occasionally (arrows) exposing the trabeculum proper (T) which shows electron optically empty spaces Blebs (B) are also observed

the cytoplasm and nucleus There was also a less pronounced disintegration of the cell organelles when compared to the endothelial cells of the corneoscleral and the uveal meshwork (Fig 8)

Schlemm s canal

The monolayer of the inner wall endothelium was disrupted in many places and the defects were located both intra and intercellularly (Figs 6 / 8) Invaginations (giant vacuoles) were frequent in areas with few defects but almost absent in areas with extensive destruction of the endothelium The nuclei of the cells appeared rounded and the cell surface towards the lumen of the canal was often uneven The cell organelles displayed more or less disintegration In the lumen of the canal cell debris blebs and free floating cells with scanty cytoplasm we e observed (Fig 8)

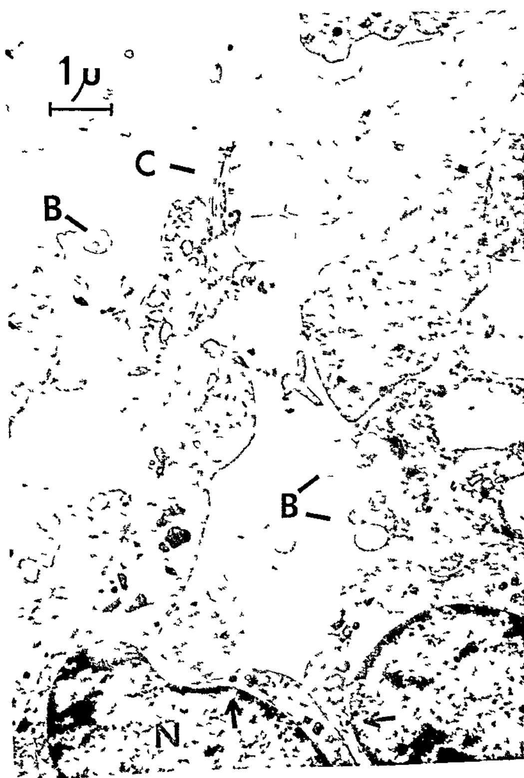
The outer wall appeared essentially normal as did the collector channels Invaginations from the scleral side were occasionally observed – al o in control eyes – but none with an opening towards the lumen of the canal

Preliminary observations on the healing process

In the three eyes fixed at 1 3 and 10 days after the period with high eye pressure there was still cell damage in the trabecular meshwork and debris in the i tertrabecular paces The conspicuous finding concerned the inner wall of Schlemm s canal With SEM the surface towards the canal no longer had any easily detectable defects However TEM revealed that the previous defects in the inner wall lining had been plugged by cytoplasmatic protrusions of the cells in the underlying endothelial meshwork – where furthermore the extracellular fibrillar and ground substance had reappeared (Fig 9)

Discussion

The experiments reported here demonstrate that an artificial elevation of the IOI caused by infu ion of fluid into the anterior chamber increases the facility of outflow and creates marked changes in the ultrastructure of the chamber angle tissue The normal appearance of the control eyes which had received the same v lume rate of infusion indicates that the effects observed were not due to the chemical composition of the perfusion fluid but that they were due t the high pre sure or the resulting high flow rate through the chamber angle tissue


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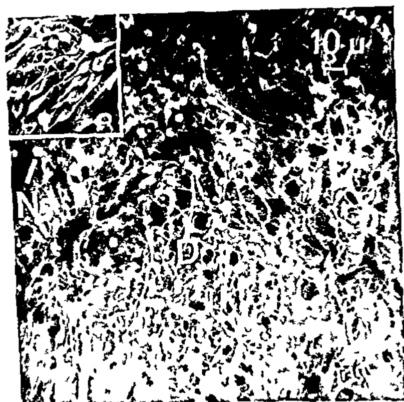


Fig 6
Inner wall of Schlemm s canal – survey Numerous defects (D) in the fitted carpet of the endothelial cells are observed Through some defects the underlying trabeculum can be seen The endothelial nuclei (N) appear rounded Insert corresponding area from a *control eye* The preparations were a r dried with ethanol

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Fig 5
Detail of endothel al cells in the trabecular meshwork. Numerous blebs (B) are seen, some lectron optically empty and some containing granular material Collagen fibrils are bser ed (C) The nucleus (N) shows peripheral condensation of nuclear heterochro matin w th a clear perinuclear zone *only interrupted at the sites of nuclear* membrane pores (arrows)

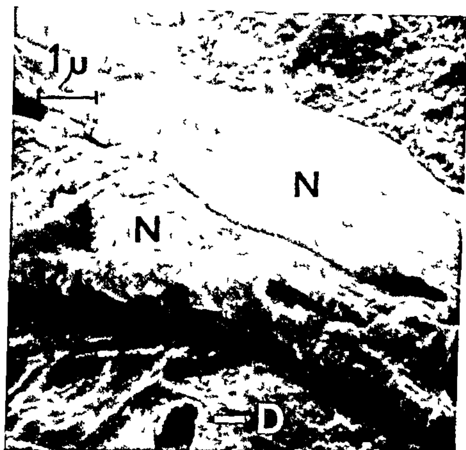


Fig. [illegible]
Detail of inner wall endothelium. Two cells with nuclei (N) are observed. The cell surface is often uneven. *Below is a defect (D). The preparation was air dried with ethanol*

Facility of outflow

Barany & Scotchbrook (1954) working with enucleated cattle eyes reported an increase of facility with perfusion time at normal IOP. The increase appeared *to be caused by the washing out of a substance from the outflow passages.* The increase of facility in the control eyes of the present study (which is in good agreement with earlier findings on vervet monkeys, Barany 1964) may have been caused by such a washing out. A tendency to a large, rather sudden facility increase at constant high IOP was observed by Bill (1971). In the present study *gross facility increased on an average of about 350 % in the eyes with high IOP* while in the control eyes the facility increase was less than 40 %. In the former eyes the endothelial meshwork and the inner wall endothelium of Schlemm's canal was disrupted in places while in the control eyes the structure of the

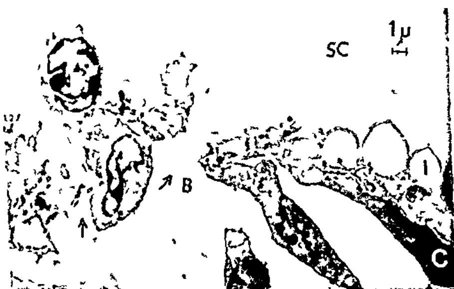


Fig 8
Inner wall of Schlemm's canal (SC) Large defects in the endothelial lining are observed (arrows) with a bleb (B) passing Note the difference in density between the endothelial cell (E) and the cells of the endothelial meshwork (C) Invaginations are marked with I

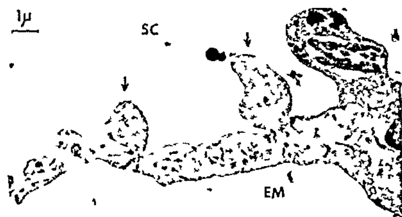


Fig 9
Inner wall of Schlemm's canal (SC) 3 days after IOP elevation Cells in the underlying endothelial meshwork (EM) are plugging the defects of the endothelial lining (arrows)

chamber angle appeared normal Other authors investigating the effects of high IOP in morphological studies (Johnstone & Grant 1973 Grierson & Lee 1973 Grierson 1974) gave no information about facility changes Those investigating the effects of high IOP on facility alone (*see* Armaly 1960 Levene & Hyman 1969 Ellingsen & Grant 1971) reported no change or decrease of facility They however were using experimental conditions (e g stepwise IOP alterations short duration of IOP elevation or *in vitro* determinations) differing from those of the present study

When calculating facility it was assumed that the resting pressure (without external inflow) was the same throughout the experiment In fact the resting pressure would probably sink a few mmHg due to decreasing aqueous humor production (Barany 1963 Bill 1971) and decreasing resistance to outflow However compared to the degree of the artificial elevation of IOP this decrease of the resting pressure was negligible When explaining the large increase of the outflow facility several factors must be considered Thus changes in intraocular volumes can be expected to be small and transient (McEwen 1972) Outflow through uveo scleral routes was probably pressure independant in the actual pressure range (Bill 1967) Deepening of the anterior chamber also seemed to be of minor importance In the actual chamber depth range the facility increased less than 50 % in enucleated human eyes according to data by François et al (1956) whereas van Buskirk & Grant (1973) using a lens depression apparatus on enucleated rhesus monkey and human eyes found an approximately 100 % increase of the outflow facility when doubling the normal chamber depth Ellingsen & Grant (1971) using a principally similar perfusion technique as in the present study demonstrated by stepwise IOP raising a progressive deepening of the anterior chamber but no change in the facility in the IOP range of 20–50 mmHg on human enucleated eyes In the present study there must thus have been a very considerable real change in the resistance to flow through the chamber angle The explanation for this change will be discussed below

Morphology

Johnstone & Grant (1973) Grierson & Lee (1975) and Grierson (1974) have reported widening of the trabecular spaces and a narrowing of Schlemm s canal at high IOP These investigators used 50 mmHg as the highest IOP for a period of 2½ and 1 hour respectively but did not observe any damage to the normal structure of the chamber angle tissue at light microscopic (Johnstone & Grant 1973) or TEM levels (Grierson & Lee 1973 Grierson 1974) In the present study such damage was evident but no obvious widening of the trabecular spaces or narrowing of the canal of Schlemm was noted The endothelial meshwork

and the inner wall endothelium of Schlemm s canal were disrupted in many places In enucleated human and monkey eyes about 75 % of the total resistance to the aqueous humor outflow is located between the anterior chamber and the lumen of the canal (Grant 1958 Moses 1971 Peterson & Jocson 1974) A rise in the facility of 350 % as observed in the experiments reported here is equivalent to a reduction in the outflow resistance by approximately 75 % Elimination of practically all the resistance between the anterior chamber and the canal of Schlemm could thus explain the effect observed on the facility of outflow The marked morphological changes found in this region indicate that this is a realistic hypothesis Elimination of this resistance would eliminate the pressure gradient tending to press the inner wall against the outer wall of Schlemm s canal which would explain why no compression of the canal was seen in the present experiments

The occurrence of blebs in the chamber angle has not been reported before perhaps because they are known to reflect poor fixation However they are also known to occur in other environmental stre s situations such as stagnant flow or hypoxia and bleb formation has been considered as an important cause of the no reflow phenomenon in brain infarction (Chiang et al 1968 Willms Kretschmer & Majno 1969) and as one of the most important mechanisms in the formation of thrombosis (Shimamoto 1972) The blebs probably arise from the cytoplasm of abnormally hydrated endothelial cells We have occasionally observed them in enucleated human eyes but not in such abundance as in the present material Whether they may occur under other conditions (for example hypoxia of the aqueous humor) and possibly plug the outflow channels is an open question

The matter of the perfusion pressure at fixation demanded concern. In the present study perfusion with fixative was done at the initial spontaneous pressure level The intention was to avoid structural alterations such as depletion of invaginations (giant vacuoles) by too small a pressure gradient between the anterior chamber and Schlemm s canal (Johnstone & Grant 1973) or artefactual breaks of the progressively hardening tissue by too large a pressure gradient.

The healing process after a period with high eye pressure has so far only been incompletely investigated since only three eyes were studied morphologically after 1 3 and 10 days respectively Nevertheless it is interesting that the normalization of the outflow facility had its morphological counterpart namely the plugging of the defects of the inner wall endothelium and the reappearance of the extracellular substance in the endothelial meshwork That other cells can help the endothelial cells to build up the inner wall has previously been demonstrated by Vegge (1967)

Since at least some cases of open angle glaucoma seem to be characterized by

accumulation of an unknown material in the endothelial meshwork it would seem logical to try and wash out this material or to create new routes for the aqueous humor outflow by intermittent IOP elevations short enough not to severely affect the blood supply to the eye However the present experimental results in normal eyes do not encourage such treatment attempts since the increase in facility was short lasting

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accumulation of an unknown material in the endothelial meshwork it would seem logical to try and wash out this material or to create new routes for the aqueous humor outflow by intermittent IOP elevations short enough not to severely affect the blood supply to the eye However the present experimental results in normal eyes do not encourage such treatment attempts since the increase in facility was short lasting

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TRANSMISSION OF ACUTE TOXOPLASMA INFECTION

The survival of trophozoites in human tears saliva and urine and in cow s milk

BY

MATTI SAARI and SIMO RÄISÄNEN

To expla n the transmission of toxoplasmosis it has been suggested that the trophozoites might not survive outside the body but that cysts oocysts and transplacental infection transmit the disease In order to evaluate the possibility that the proliferative form transmits the disease trophozoites were preserved in human tears saliva, and urine and in pasteurized cow s milk at 4°C and for isolation inoculated daily into healthy toxoplasma free mice i p It was observed that trophozoites remained infectious in ex creti ns for several days in tears for 4 days in saliva for 5 days in urine for 7 days and in milk for 6 days Results suggest that trophozoites can survive in excretions outside the body long enough to transmit the disease.

Key u di excretions – infectious diseases – tears – *Toxoplasma gondii* t xoplasmosis – transmiss on – trophozoite – uveitis

In congenital toxoplasmosis the eyes often are involved Langset et al (1970) found a statistically significantly higher number of positive dye test titers in blind children in Norway From the neurosurgical service in Copenhagen Bech & Mellemgaard (1968) reported that 33 % of the congenital anomalies of the eye

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The excretions Fresh tears saliva and urine from a healthy man and past eurized cow s milk were used in the study

Performance of the test For every inoculation a 5 ml closed test tube contain ing 0 2 ml of mouse ascites with free toxoplasma trophozoites and 1 8 ml of excretion was maintained at 4 C The survival of trophozoites in test tubes containing saliva urine or milk was tested up to 14 days and during the first week daily inoculations were done by injecting each excretion separately into *four 25–30 g* toxoplasma free male NMRI *strain albino mice i p The inoculation* of trophozoites in tears was performed in a similar manner after 2 and 4 days After inoculation the *mice were* followed until as clinical symptoms of acute toxoplasma infection their skin became roughened and they developed difficul ties in moving they were then killed and the ascites was studied under a light microscope The isolation was considered to be positive if the mice showed clinical symptoms of acute toxoplasma infection and if the ascites contained intra and extracellular parasites Positive isolation was confirmed with a new inoculation into healthy mice

Results

The trophozoites remained infectious in tears for the entire follow up period of 4 days (Table I) The *trophozoites remained infectious in human saliva for* 5 days but the inoculation of trophozoites which had been preserved in the

Table I
Effect of preservation time and source of excretion on infectivity of *Toxoplasma gondii* tr phozoites as *tested in mice i p* Positive isolation + negative isolation –

Excretion	Preservation time (days)							
	1		3	4	5	6	7	14
Tears		+		+				
Saliva		+	+	+		–	–	–
Urine	+	+	+	+		+	+	–
Milk		+	+	+	+		–	–

are due to toxoplasmosis In southern Finland 22 7 % of cases of posterior uveitis were attributed to toxoplasmosis (Juurikkala 1961) and 70 % of acute posterior uveitis in England were considered to be toxoplasmic in origin (Perkins 1961) Ophthalmologists are probably more aware of this disease than are other medical specialists (Scott 1974)

The diagnosis of ocular toxoplasmosis may be difficult because of the problem of isolating the organ sm and because organisms residing in the retina may cause only little stimulation of antibody production (Zscheile 1964) Pyrimethamine sulpha and steroids are used to treat the ocular form of the disease However there is no good evidence for the efficacy of any of these therapies (Scott 1974) and the present methods of treatment are of limited value against the cystic form of toxoplasma (Quinn & McCraw 1972) which may persist in the tissue for the life of the patient (Remington & Cavanaugh 1965) Therefore efforts should be concentrated on preventing the transmission of the disease and especially the congenital form of the infection

It has been generally accepted that transmission of toxoplasmosis to man *occurs transplacentally through the consumption of raw meat containing* tissue cysts or by ingestion of food contaminated by oocysts of cat faeces (Dubey et al 1970 Janitschke 1971 Krogstad et al 1972 Frenkel & Dubey 1972 Quinn & McCraw 1972 Scott 1974)

In acute infections of toxoplasma the proliferative form the trophozoite (Jacobs 1963 Jacobs & Hartley 1964) causes parasitaemia. The trophozoites are then widely distributed throughout the body and liberated in serous exudates faeces urine saliva sputum nasal and conjunctival secretions vaginal discharges semen *and milk (Christie 1969 French et al 1970 Janitschke 1971)* The hypothesis that trophozoites cannot survive in excretions outside the living tissue long enough to transmit the infection (Hutchison 1967 Christie 1969 Jacobs 1970 Dubey et al 1970) has not been proved experimentally

The aim of this study was to test the survival of trophozoites in human excretions and cow s milk and to evaluate the possibility that the proliferative form transmits the infection

Material and Methods

Source of toxoplasma trophozoites The RH strain of *Toxoplasma gondii* was used throughout the study as the test organism This strain is highly virulent and is lethal to mice It was maintained by serial passage through 25–30 g male strain albino mice (Orion OY Pharmacological and Chemical Laboratories)

The excretions Fresh tears, saliva and urine from a healthy man and pasteurized cow's milk were used in the study

Performance of the test For every inoculation a 5 ml closed test tube containing 0.2 ml of mouse ascites with free toxoplasma trophozoites and 1.8 ml of excretion was maintained at 4°C. *The survival of trophozoites in test tubes* containing saliva, urine or milk was tested up to 14 days and during the first week daily inoculations were done by injecting each excretion separately into four 25–30 g toxoplasma free male NMRI strain albino mice i.p. The inoculation of trophozoites in tears was performed in a similar manner after 2 and 4 days. After inoculation the mice were followed until, as clinical symptoms of acute toxoplasma infection, their skin became roughened and they developed difficulties in moving; they were then killed and the ascites was studied under a light microscope. The isolation was considered to be positive if the mice showed clinical symptoms of acute toxoplasma infection and if the ascites contained intra- and extracellular parasites. Positive isolation was confirmed with a new inoculation into healthy mice.

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saliva for 6 days or longer did not cause acute toxoplasmosis in mice (Table I) A positive isolation was observed after inoculation from the test tubes containing human urine for up to 7 days but the trophozoite did not remain infectious in urine for 14 days (Table I) In milk the trophozoite stayed infectious for up to 6 days after which time it did not infect the mice (Table I)

Discussion

In human beings transplacental transmission of toxoplasmosis to the foetus occurs only during the acute stage of infection and therefore acute toxoplasmosis should be avoided during pregnancy The results of the present study suggest an additional route of transmission of toxoplasma infection by the trophozoites in excretions (Fig 1)

Toxoplasma gondii actively penetrates cell cultures within 15–30 sec (Bommer 1969) The results of this study showed that trophozoites remain infectious in

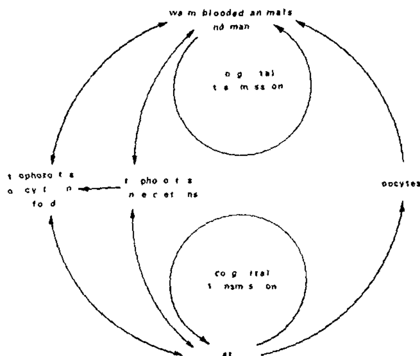


Fig 1
A suggested scheme for the transmissio oplasmosis

human tears saliva and urine long enough to cause acute illness if inoculation happens Congenital transmission occurred in ewes infected by oral intranasal or conjunctival routes (Jacobs & Hartley 1964) During the acute stage of infection the trophozoites could be transmitted in man venereally by mouth to mouth route and by secretions The results of this study suggest also that the trophozoites survive in human excretions long enough to reach the laboratory for isolation and need not be inoculated into mice immediately On the other hand the laboratory personnel should also be on guard against trophozoites in older specimens

An acute stage of toxoplasma infection occurs in 10–90 % of domestic animals at least once in their life Mayer (1962) studied 304 cows and isolated *Toxoplasma gondii* from the retinas of 74 animals In the United States the proportion of animals with toxoplasma antibodies varied between 14 and 43 per cent (Krogstad et al 1972) Part of the livestock is continually in an acute stage of infection and liberates trophozoites in excretions and secretions Many case histories have been presented showing acute infections in humans following close contact with infected domestic and farm animals (Wende & Dienst 1961) Basic hygienic precautions should be taken in handling domestic and pet animals especially by pregnant women

As the oocysts of the cat faeces are infectious for several months in moist environments and are resistant to chemicals (Dubey et al 1970) disposable gloves should be used while cleaning cat litterpans Pregnant women should avoid stray cats

Nobody should eat raw meat because it may contain toxoplasma cysts (Krogstad et al 1972) On the other hand the results of this study showed that trophozoites survive in milk for 6 days Trophozoites do not, however survive the process of pasteurization Pasteurized milk is therefore safe to use, but contaminating it, as well as other food with infective excretions should be guarded against

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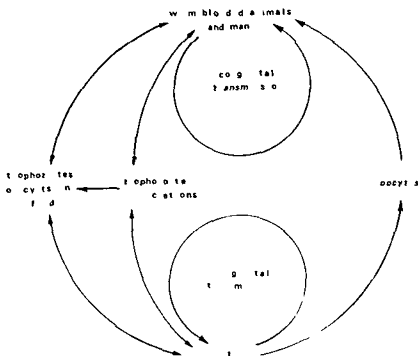


Fig 1
A suggested scheme for the transmission of toxoplasmosis

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THE ΔP/ΔV RATIO

New method of eye measurement

BY

JOSEPH STEPANIK

In 11 normotensive eyes numerous ΔV were calculated from corneal applanations of different sizes To each ΔV the corresponding ΔP was measured by Goldmann s applanation tonometer Friedenwald s coefficient K as deri ed from the results of this study (K) was in accordance with the manometrics on living eyes of Prijot Weekers and Ytteborg and de f nitely smaller than that given by differential tonometry (K_t) performed on the same eye

Key wo d aqueous dynamics – ΔP/ΔV ratio – tonometry

The possibility and necessity of measuring the ΔP/ΔV ratio in living human eyes was signalized by Friedenwald (19 4) when Grant proceeded to use an electromanometer with a train gauge unit on rabbit eyes But it was not until 1959 that Prijot & Weekers used this equipment for the first time on two human eyes *in vivo* followed by measurements on 12 eyes by Ytteborg in 1960 and on four eyes in 1961 by Prijot In local or general anaesthesia one or two cannulas were introduced into the anterior chamber of the patient s eye and the pressure

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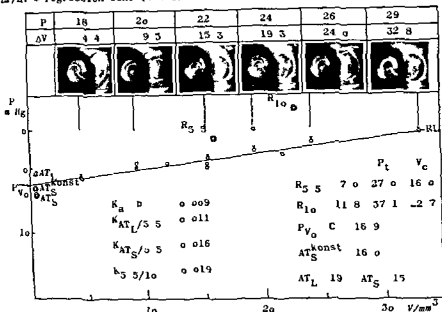


Fig 1

Abbreviations Radius of corneal curvature (r) Reading of Goldmann's applanation tonometer on patients in supine (AT_L) and in sitting position (AT_S and AT_S konst) Scale reading of the Schiøtz tonometer with 5 5 g ($R_{5.5}$) or 10 g weight (R_{10}) and the corresponding pressure (P_t) and volume of corneal indentation (V) during tonometry (Friedenwald 1955) Intraocular pressure (P) after intake of applanation volume (V) Friedenwald's coefficient k as derived from log P/V (k) Regression line (RL) and its *intercept of the ordinate* (P)

Pressure/volume measurements on sitting patient Applanation tonometry (AT) was repeated every 2 min until a constant reading was achieved (AT_S konst) After this Goldmann's applanation tonometer was adapted to different P adding 2 4 6 etc mmHg to the value AT_S konst and adjusted to the left part of the cornea The remaining free cornea was applanated by a plexiglass plate as much as was necessary to increase the intraocular pressure from AT_S konst to the chosen value P A flash light photograph was taken (Fig 1) showing the s like figure of Goldmann's applanating prism and the fluorescein ring surrounding

changes (ΔP) made by injections of known volume (ΔV) using a microsyringe, were recorded by the electromanometer Each of those manometries was preceded by *clinical differential tonometry* with Goldmann s applanation tonometer in sitting position versus Schiotz tonometer in supine position Accepting Friedenwald s formula

$$k = \frac{\log \Delta P}{\Delta V} \qquad (1)$$

the slope of the regression line was determined from the manometries (k_s) and from the differential tonometries (k_t) In all these studies k_s averaged definitely smaller than k_t

$$k_s \quad k_t = 0\,6 \quad 1\,0 \qquad (2)$$

Besides the explanations given by the authors the question arose how far if not exclusively *this discrepancy in the k values might be caused by the reaction* of the living eye to the introduction of cannulas into the anterior chamber

Therefore to eliminate this trauma the $\Delta P/\Delta V$ ratio was studied with the following new method The intra ocular pressures were measured exclusively by Goldmann s applanation tonometer and the volumes (V) were displaced into the eye by corneal applanations

Method

The radius of the corneal curvature (r) was determined by the ophthalmometer Eyes with corneal astigmatism of more than 1 diopter were eliminated from this study

Tonometries in supine position When the patient had been in the supine position for 10 min the intraocular pressure was measured three times at the same sequence (i e 9 measurements/eye)

Applanation tonometer (AT_L)
Schiotz tonometer 5 5 g (R)
Schiotz tonometer 10 g (R_{10})

The values used correspond to the average of each triplet reading

Table I
Results of differential tonometry

Eye no	AT_L	$R_{5.5}$	R_{10}	AT_S	k_t				
					5 5/10	AT_S/5 5	AT_S/10	AT_L/5.5	AT_L/10
1	24	5 0	10 7	22	0 013	0 013	0 013	0 011	0 011
2	24	4 8	11 0	21	0 009	0 016	0 013	0 012	0 010
3	17	6 1	10 5	16	0 027	0 018	0 021	0 018	0 020
4	17	7	9 7	16	0 034	0 028	0 030	0 019	0 022
5	16	7 0	12 2	10	0 016	0 028	0 024	0 017	0 015
6	16	7 5	12 0	17	0 024	0 011	0 015	0 013	0 016
7	17	7 6	13 2	15	0 013	0 014	0 014	0 012	0 012
8	19	7 0	11 8	15	0 019	0 016	0 018	0 011	0 012
9	0	5 0	9 7	15	0 022	0 026	0 026	0 018	0 019
10	14	6 8	11 5	13	0 020	0 020	0 019	0 020	0 020
11	21	4 5	8 3	20	0 040	0 020	0 026	0 019	0 024
x	18 5			16 0	0 0214	0 0191	0 0199	0 0154	0 0164

Reading of Goldmann's applanation tonometer on supine patient (AT_L) and on sitting patient (AT_S) in mmHg Reading in scale units of the Schiotz tonometer when using the 5 5 g ($R_{5.5}$) or 10 g weight (R_{10}) Friedenwald's coefficient k as derived from possible couples of the above tonometer readings (k_t) Each reading corresponds to the average of three measurements

the corneal applanation produced by the plexiglass plate. The former proves that the intraocular pressure was properly raised to the chosen P and the latter is used for calculating the volume of corneal applanation V_a applying the formula for spherical segment and taking into account the radius of the outer corneal curvature (r) and the diameter of the corneal applanation as seen from the photograph (Stepanik 1968a).

The equipment used in this study corresponds to that of the applanation rheometry (Stepanik 1966, 1968b). A 10 × 10 mm plexiglass plate is adjusted in a vertical position to Goldmann's applanation tonometer by a rigid arm in such a manner that the plane of the tonometer applanation body and that of the plate form an open angle of 130° against the cornea. The applanation body can be adjusted to the cornea alone or, by further advancement of the slit lamp towards the eye, together with the plexiglass plate. The s-like *fluorescein figure* of the applanation tonometer as well as the fluorescein ring surrounding the additional corneal applanation are seen simultaneously through the viewer of a mirror reflex camera and a picture is taken when adjustment is correct by releasing a trigger mounted on the handle of the slit lamp. This study was performed on 11 eyes.

Results

The differential tonometry

From the readings given by the differential tonometry (AT_L), $R_{5.5}$, R_{10} and (AT_S) the various possible K_t's were taken from Friedenwald's nomogram (1955) as follows:

$K_{5.5/10}$ from readings of the Schiotz tonometer $R_{5.5}$ and R_{10}
$K_{ATS/5.5}$ from AT_S versus $R_{5.5}$
$K_{ATS/10}$ *from* AT_S *versus* R_{10} *and finally*
$K_{ATL/5.5}$ and
$K_{ATL/10}$ from AT_L versus $R_{5.5}$ and R_{10}.

The results (Table I) show that on the average K_t is highest when using the Schiotz tonometer alone ($K_{5.5/10} = 0.0214$), somewhat smaller when the Schiotz tonometer readings are correlated to Goldmann's tonometer readings in a sitting patient ($K_{ATS/5.5} = 0.0191$, $K_{ATS/10} = 0.0199$) and lowest when derived from Schiotz versus applanation tonometer taken on patients *in a supine position* ($K_{ATL/5.5} = 0.0154$, $K_{ATL/10} = 0.0164$).

Table II

Results of pressure/volume measurements

Eye no	n	k_a	P_{V_0}	AT_S t	P_{V_0} AT_S tt
1	7	0.0085	16.94	16	0.94
2	6	0.0060	18.61	18	0.61
3	10	0.0128	16.35	16	0.35
4	4	0.0139	15.31	13	2.31
5	8	0.0170	9.71	10	−0.29
6	5	0.0134	11.10	12	−0.90
7	7	0.0111	16.47	16	0.47
8	21	0.0088	16.90	16	0.90
9	13	0.0119	16.18	15	1.18
10	25	0.0081	1[illegible].56	13	−0.44
11	7	0.0160	17.76	17	0.76
x	10	0.0116	15.[illegible]6	14.73	0.53

n = number of measurements/eye

k regression coefficient (b)

P_V intercept of the ordinate by the regression line (C)

Table III

	n	k_t	k_r	k_a
Ytteborg	10	0.0?09	0.0130	
Prijot	6	0.0190	0.0116	
Stepanik	11	0.0191		0.0116

Comparison of Friedenwald's coefficient k (x) as derived from differential tonometry (k_t) and from volume/pressure measurements by electromanometer versus microsyringe injections (k_r) or by applanation tonometer versus corneal applanations (k_a)

The pressure/volume measurements

In each of the 11 examined eyes 5–25 (n = 10) pressure/volume measurements were performed. The results per eye were put into a scattergram (Fig. 1) with V_a (applanation volume in mm³) on the abscissa and log P (intraocular pressure in mmHg) on the ordinate. For the given points, a regression line was determined from

$$\log P = \log \bar{P} + b\ (V_a - \bar{V}_a) \tag{3}$$

in which the regression coefficient b corresponds to Friedenwald's coefficient K. When V_a is equal to zero the intercept of the ordinate by the regression line log C results from

$$\log C = \log \bar{P} - b\ \bar{V} \tag{4}$$

and then the regression line can also be expressed by

$$\log P = \log C + b\ V \tag{5}$$

or instead of using C and b terms corresponding to their meaning in this study by

$$\log P = \log P_{V_0} + K_a\ V \tag{6}$$

P_{V_0}, the intraocular pressure without additional corneal applanation, should correspond to slightly less than the value measured by the applanation tonometer at the beginning (AT_{start}). Taking the average from 11 eyes P_{V_0} was 15.26 and AT_{start} 14.73 mmHg. Although moderately significant ($P < 0.05$) *the small difference $P_{V_0} - AT_{start} = 0.53 \pm 0.28$ ($\bar{x} \pm s_{\bar{x}}$)* may be neglected (Table II). Thus the calculated regression line gives sufficient indication on the ordinate of the intraocular pressure of the untouched eye.

K_a, i.e. Friedenwald's coefficient K as derived from the pressure/volume measurements in this study, was averaged at 0.0116 (Table II) and definitely smaller than all K_t's as derived from clinical differential tonometry.

In Table III the given average difference of both K values is compared with those of Ytteborg and Prijot. In accordance with their report, from the different K_t values of Table I the average of K_{t1} was opposed to K. There is an almost unbelievable conformity in the results given by the three authors.

Thus this study not only supports the findings of Prijot, Weekers and Ytteborg, it also eliminates the reaction of the eye to puncture of the anterior chamber as a possible cause of the given difference ΔK, and it clearly demonstrates that the true ΔP/ΔV ratio can be determined *in vivo* by a new clinical procedure applicable to every eye.

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THE FRIEDMANN VISUAL FIELD ANALYSER TESTED ON NEURO OPHTHALMOLOGICAL CASES

BY

H BYNKE and L NORDENFELT

The Friedmann Visual Field Analyser has been tested on 108 visual fields of which 67 had neuro ophthalmological defects and 41 were normal according to the kinetic Goldmann perimeter which was used as a control method For an exact evaluation of the extent of the defects and for follow up studies the analyser was found to be less suitable The most obvious advantage of the instrument is that it may be operated even by an untrained assistant Used at one single luminance level of 0 ? log units above the one recommended according to the age of the subject it was found to be a rapid and fairly reliable screening method If three fields with enlarged blind spots were excluded there were four relative hemianopias which escaped detection i e 6 % false negative fields In these cases the analyser happened to detect the hemianopia of the fellow eye There were also a number of false positive results

Key word Friedmann Visual Field Analyser – kinetic Goldmann perimeter – bitemporal hemianopia – homonymous hemianopia – central scotoma – paracentral scotoma – enlarged blind spot

The Friedmann Visual Field Analyser is a static multiple stimulus instrument for examination of the field inside 25 degrees (Friedmann 1966 Bedwell 1967) Two three or four stimuli are presented in groups until a total number of 46 stimuli have been tested The light source is a Xenon flash tube with a very short presentation time

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Acknowledgement

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six enlarged blind spots in four relative hemianopias in 41 and absolute hemianopias in 12 fields The hemianopia was bitemporal in 16 cases and homonymous in 15 The homonymous defects included superior and inferior quadrantanopias

Twenty one fields from the patients and all 20 from the healthy subjects were normal according to Goldmann perimetry

Methods

All the subjects were examined both with the kinetic Goldmann perimeter which was used as a control method and with the Friedmann Visual Field Analyser

At the Goldmann perimetry at least two and in the majority of the cases with defective fields more than three objects were used (Enoksson 1965) Special attention was devoted to exploring the central field this being important, since the analyser examines the field only inside 25 degrees from the fixation point

In the majority of the cases the Goldmann perimetries were performed by the authors or by trained assistants whose results were then checked by the authors Only in a few cases with large defects was the whole procedure left to the assistants

The estimation of the Goldmann charts was based on clinical judgement In addition in 11 cases with bitemporal and six with homonymous hemianopia the Goldmann fields of the right eyes were quantified by cutting out the areas inside the 0 3 isopter from the charts and weighing them In this way a method was obtained for quantitative comparison of the results of the two methods

The examination with the analyser was started after 5 min of dark adaptation Firstly the luminance recommended according to the age of the subject was used i e a filter setting of 2 0 for ages up to 40 years 1 8 between 41 and 50 etc (Friedmann 1966 Bedwell 1967) Then the luminance was raised stepwise by 0 2 log units until all stimuli had been detected or until maximal luminance had been tried

Unlike the perimeter the analyser has a very standardized programme Therefore in the majority of the cases this examination was left to the assistants

For the luminance recommended according to age the number of stimuli missed was found to be unexpectedly large For example one healthy subject with normal Goldmann fields missed 10 stimuli at this luminance Therefore

The luminance of the stimuli may be varied by means of neutral density filters The inventors have recommended starting the examination at a certain luminance level adapted to the age of the subject If any stimulus is missed at this level the luminance is raised stepwise and the programme is repeated until all stimuli have been detected, or, until maximal luminance has been used

The instrument has been tested on clinical cases by Lechner & Haefner (1971) who used the kinetic projection campimeter and the Maggiore perimeter as control methods These authors found the analyser well suited to detecting field defects of various types However several of them seem to have been large which may have contributed to the good results Demailly et al (1973) found the analyser able to detect glaucomatous defects which had not been revealed by the kinetic Goldmann perimeter That this comparison favoured the analyser was possibly due to the fact that the full capacity of the Goldmann perimeter was not utilized since only one object had been used

It is evident that the results of a comparison between two instruments depend on the type and size of the field defects and on the manner in which the instruments have been used

In the present work the analyser has been tested on neurological field defects several of them slight ones The control method was the quantitative kinetic Goldmann perimetry

Material

The material consisted of 108 visual fields from 58 subjects 29 male and 29 female aged between 8 and 69 years

Forty eight subjects were patients with diseases of the central nervous system as tumours vascular lesions and multiple sclerosis At the time of the present investigation from Oct 1973 to Feb 1974 or previously they were treated as in patients in various clinics of the University Hospital in Lund and were thoroughly examined by neuro radiological and other methods In the tumour cases the diagnoses were verified at neurosurgical interventions

The material also included a group of 10 healthy subjects belonging to the staff of the Eye Clinic The reason why this group was small is that detailed examinations of healthy eyes have been performed by previous authors (Bedwell 1967, Greve 1971 and 1973)

Sixty seven out of the 108 visual fields were defective according to Goldmann perimetry There were central scotomas in four fields paracentral scotomas in

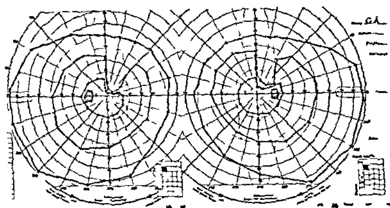


Fig 1 a
Woman aged 45 Homonymous superior quadrantanopia due to vascular lesion in temporal lobe

at maximal luminance was there a significant negative correlation This may be explained by the fundamental differences between the two methods

In three cases of congruous homonymous hemianopia, right sided in two cases and left sided in one the analyser gave the information that the hemianopia was incongruous since the number of stimuli missed was larger in the left field (av 11 at +0 9 log units) than in the right (av 8)

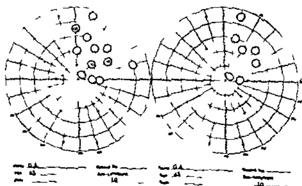


Fig 1 b
Same case as in 1 a Good correspondence of results of analyser and perimeter

in this series the results were quantified by stating the numbers of stimuli missed when the luminance was raised 1) 0.2 and 2) 0.6 log units above this level and when it was 3) maximal

In nine cases of bitemporal and two of homonymous hemianopia belonging to 10 patients with pituitary adenoma and one with a suprasellar meningioma the two examinations were repeated once (6 cases) twice (3 cases) or three times (2 cases) In seven cases the first examinations were made before the neurosurgical intervention and the subsequent ones afterwards In the other four cases all the examinations were performed in the initial post operative period The interval between the first and last examinations was less than 4 months in all the cases

Results and Discussion

If various luminances were tested the analyser examination was found to be about as time consuming (15–32 min) as the quantitative Goldmann perimetry (20–35 min) but in spite of this less informative It is true that in the majority of the cases the appearance of the fields recorded with the analyser was similar to that recorded with the perimeter (Figs 1a and b) but in many other cases it was difficult to evaluate the extent of the defects on the basis of the charts of the analyser (Figs 2a and b) Furthermore this performance did not guarantee that all defects were detected For example in some cases of relative hemianopia the analyser detected the defect only in one field (Figs 3a and b)

Greve (1971) demonstrated that a relative bitemporal hemianopia escaped detection by the analyser Therefore he recommended starting the examination from the infraliminal luminance level We agree but must add that such a performance would be still more time consuming and would detect numerous false positive defects which must then be re examined using other methods

As expected the numbers of stimuli missed were larger in large defects than in small ones For example in the fields with relative hemianopia the mean numbers of stimuli missed were 14 (+0.2 log units) and six (maximal luminance) and in the fields with absolute hemianopia 22 and 19 respectively

A quantitative comparison between the numbers of stimuli missed and the sizes of the central perimeter fields was performed in the 1[illegible] fields with hemianopia in which the central fields were cut out from the Goldmann charts Spearman s rank correlation coefficient (r) was calculated as –0.38 (+0.2 log units) –0.45 (+0.6 log units) and –0.55 (maximal luminance) Consequently only

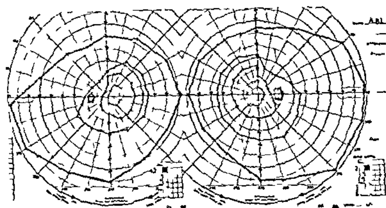


Fig 3a
Woman aged 51 Relative bitemporal hemianopia due to pituitary adenoma

This seems to be another proof that the exact extent of the defects is difficult to evaluate on the basis of the charts of the analyser

Because of its standardized and reproducible programme the analyser has been supposed to be well suited for follow up studies (Friedmann 1966 and 1969 Demailly et al 1973) In the 11 cases of tumours in the sellar region in which both examinations were repeated 27 occasions were produced for a comparison

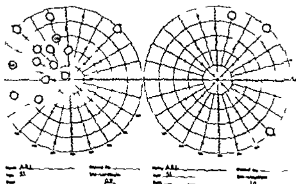


Fig 3b
Same case as in 3a The analyser recorded the defect only in the left field In the right the number of stimuli missed and their depths were too small to be definitely pathological

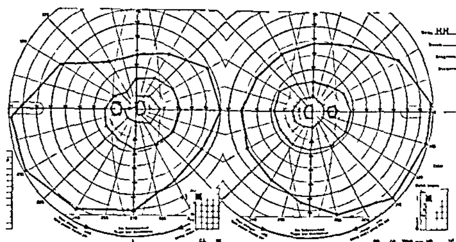


Fig 2 a
Woman aged 38 Relative bitemporal hemianopia due to pituitary adenoma

Friedmann (1969) described 74 cases of homonymous hemianopia in which the analyser showed an unexpectedly large incongruity On the basis of these results he suggested that congruity is a poor localizing sign We believe that the results are more likely to be an error arising from the method The multiple stimulus method the relatively small number of stimuli and the fact that six stimuli are situated in the vertical meridian may explain the false incongruity

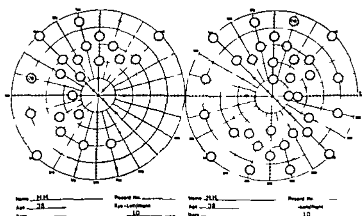


Fig 2 b
Same case as in 2 a The extent of the defects is difficult to evaluate from these analyser charts

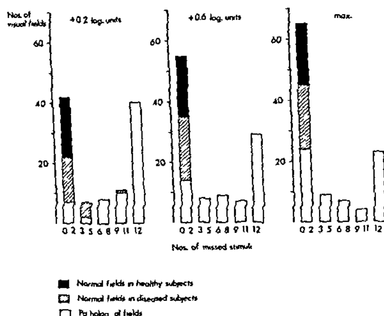


Fig. 5
Numbers of stimuli missed in the whole material at three luminance levels. See text

Therefore we applied the hypothesis that a field is defective if more than two stimuli are missed at this luminance.

In six out of the 21 normal fields from the patients there were more than two stimuli missed and in seven out of the 67 defective fields there were fewer than three stimuli missed at this luminance. Consequently, if we accept the hypothetic normal value there were 6/41 (15%) false positive fields and 7/67 (10%) false negative ones in this material (Fig. 5).

At higher luminance levels the false positives disappeared and the false negatives increased. For example at +0.6 log units there were 14 and at maximal luminance 24 defective fields with less than three stimuli missed (Fig. 5).

Thus for screening a luminance of 0.2 log units above the one recommended according to age was found to be the most suitable.

The false positive fields belonged to the patients with localized cerebral lesions. The explanation of the phenomenon is obscure but may be either a reduced ability to cooperate or that the multiple stimulus method also records psycho-visual disturbances (Greve 1969).

PERIMETER \ ANALYSER	+	0	−
+	9	5	2
0	2	6	0
−	2	1	0

+ improved
0 unchanged
− deteriorated

Fig 4

Comparison of results of analyser and perimeter at repeated examinations in 11 cases On 15 occasions the correspondence of the methods was good on 12 occasions less good or bad

of the methods on this point On 15 occasions there was a good correspondence of the results of the methods (Fig 4) On 12 occasions this was less good or even bad obviously to the disadvantage of the analyser For example on two occasions there was a definite improvement according to perimetry but the analyser showed a deterioration Thus the present results indicate that the analyser is inferior to the Goldmann perimeter in follow up examinations This cannot be attributed only to the fact that the intensity of the flash diminishes in the course of time (Greve 1973) since the intervals between the examinations were short

Because of the qualities mentioned the analyser may be operated even by an untrained assistant This seems to be the most striking advantage of the method

If much time is to be spared the examination must be restricted to one single luminance level It was of interest to evaluate the capacity of the analyser when it was used in this simplified manner since it may then fulfil the demands on a screening method

Now the question arose which luminance was best suited for such an examination As mentioned the numbers of stimuli missed were found to be too large at the luminance level recommended according to age and still larger at lower luminances At a luminance of 0 2 log units above the one recommended there were 0–2 stimuli missed in the 20 normal fields from healthy subjects (Fig 5)

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As many as 10 % false negative fields is hardly tolerable However it must be pointed out that in three fields there was only an enlarged blind spot due to papilloedema These cases would already have been diagnosed with the ophthalmoscope Greve (1973) and Demailly et al (1973) mentioned that the analyser is not suitable for detecting moderately enlarged blind spots because of the position and limited number of the stimuli

The other false negatives consisted of four fields with a relative hemianopia Consequently it would be more justified to calculate the false negative fields as 4/67 (6 %) In this material even these fields happened to be of little practical importance since the hemianopias of the fellow eyes were detected by the analyser

Finally it should be mentioned that the analyser has been found to be usable for examination of some children who do not cooperate for kinetic perimetry Such cases have not been included in this material

Conclusions

1 If various luminances are tested with the Visual Field Analyser this method is about as time consuming as the quantitative Goldmann perimetry but less informative Thus for an exact evaluation of the extent of the defects and for follow up studies it is less suitable than perimetry

2 The most evident advantage of the instrument is that it may be operated even by an untrained assistant Used at one single luminance level of 0 2 log units above the one recommended according to the age of the patient it is a rapid and fairly reliable screening method

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Various types of defects were recently described by Hoyt Frisen & Newman (1973) Hoyt and co workers (1973 1974) also described such defects in glaucoma demyelinating optic neuropathy and other nerve diseases They were always associated with a corresponding visual field defect It was pointed out that a careful examination of the retinal nerve fibre layer has a great diagnostic potential

It is the aim of this article to confirm the occurrence of such ophthalmoscopically visible defects of the retinal nerve fibre layer

The cases presented here were selected because of their conspicuous changes in the retinal nerve fibre layer Criteria for abnormality were the same as those detailed by Hoyt Frisen & Newman (1973)



Fig 1

Ischem c ptic neuropathy Right eye N te the distinct upper temporal border (*short arrow*) and the more blurred lower nasal border (bracket) that enclose an area with a t tal loss of n rv fibre pattern The vessels within this area are narrow and appear naked (long arr w)

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WASTING OF NERVE FIBRES IN THE RETINA

Photographic documentation

BY

MATS LUNDSTRÖM

Local disappearance of retinal nerve fibres can be seen ophthalmoscopically as dark slits and wedges in the retinal nerve fibre layer This article documents photographically the occurrence of such defects in various diseases of the eye and the optic pathways and illustrates the diagnostic potential of the ophthalmoscopic examination of the retinal nerve fibre layer

Key words fundus photography – glaucoma – ophthalmoscopy – optic atrophy – retinal anatomy

Any disease that causes a cross section damage anywhere along the axon of a retinal ganglion cell produces axonal degeneration that occurs in both directions from the point of damage Anderson (19 3) showed in the monkey that descending atrophy started between 4 and 5 weeks after an experimental lesion of the optic nerve Ascending atrophy started 2 weeks after experimental lesion of the peripapillary area of the retina

The normal funduscopic appearance of the nerve fibre layer will change when atrophy occurs in the retinal part of the axon The normal retinal nerve fibre pattern is better seen in red free light (Vogt 1913) Defects in the retinal nerve fibre pattern are also enhanced in red free light (Hoyt et al 1972)

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The cases presented here were selected because of their conspicuous changes in the retinal nerve fibre layer Criteria for abnormality were the same as those detailed by Hoyt Frisen & Newman (1973)



Fig 1

Ischemic optic neuropathy Right eye Note the distinct upper temporal border (short arrows) and the more blurred lower nasal border (bracket) that enclose an area with a total loss of nerve fibre pattern The vessels within this area are narrow and appear naked (long arrow)

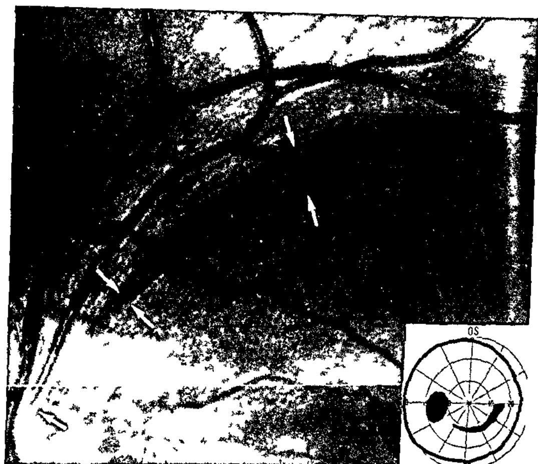


Fig. 2
Malignant arterial hypertension. Left eye. A wedge shaped nerve fibre layer defect is visible in the superior arcuate bundle. The defect can be followed from the disc margin at 1 o'clock and about 3 disc diameters distally (arrows indicate the borders).

Methods

In every case a careful direct ophthalmoscopy using a Jena Hand Ophthalmoscope was performed with and without the built in red free filter. The visual field was examined at the tangent screen (3/2000 white). The funduscopic appearance was documented with a Carl Zeiss Fundus Camera. This was provided with a built in interference filter with a maximal transmission at 480 nm and a spectral half width of 25 nm. Kodak Tri X film developed in Kodak Microdol was used. The black and white negatives were printed on Kodak Ektamatic SC paper or Ilford paper No. 4.



Fig 3

Low tension glaucoma Right eye There is a deep inferior marginal excavation at 6 to 7 o clock (black arrow) In the corresponding retinal sector no nerve fibre pattern is discernible and there is a sharp border between this area and the opaque appearance of the papillomacular bundles (broad arrows) In the superior arcuate bundle a wedge shaped defect is seen (long arrows at 11 o clock)

Case Reports

Case 1 Male aged 40 Sudden loss of vision of the right eye during hard physical work A few seconds later partial improvement occurred He was examined elsewhere 18 days after this episode The right optic disc seemed normal at this time He was examined at the Sahlgrenska Hospital 55 days after the visual disturbance At this time the right optic disc was pale and flat A total lack of nerve fibre patterns was found superior and nasal to the optic disc (Fig 1) There was also a visual field defect corresponding to the nerve fibre defect The intraocular pressure was 14 mmHg by applanation The visual acuity was 1 0 A diagnosis of ischemic optic neuropathy was made

Case 2 Male aged 34 Severe arterial hypertension was detected in November 1973 He was examined by an ophthalmologist in December 1973 and many haemorrhages and exudates were found in both fundi The arteries were attenuated There was no



Fig 4

Multifocal demyelinating neuropathy Left eye In the superior arcuate bundle multiple narrow slit like defects are seen (arrows) The superior arcuate bundle has a raked appearance The papillomacular fibres are poorly defined

oedema of the optic discs After therapy was started the funduscopic picture started to normalize In April 1974 several retinal nerve fibre defects were found bilaterally (Fig 2) Each defect had a correlated visual field defect At this time the haemorrhages

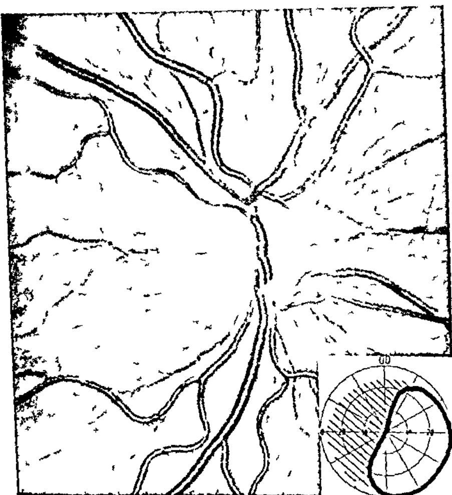


Fig 1

Opti tra t lesion R ght (A) and left (B) eye There is a partial atrophy of nerve fibres fr n th right hal f th retinas (note that the dividing line between functional and n n fun t nal p rts f the ret na runs through the centre of the macula in cases with h n nym u ual field defects) There is a c nsiderable loss of temporal f bres in the right ey A and the normal d fference between arcuate and nasal bundles is decreased. In th l ft B) the nasal nerve fibre pattern is poorly manifested but the arcuate bun lles fr m th left half f the retina have a normal appearance Note the distinct b r l f the left ptic disc ndicat ng a partial opt c atrophy

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Multifocal demyelinating neuropathy Left eye In the superior arcuate bundle multiple narrow slit like defects are seen (arrows) The superior arcuate bundle has a raked appearance The papillomacular fibres are poorly defined

Case 4 Male aged 24 There was an 18 month s history of fluctuating visual loss in the left eye A neurological examination disclosed nothing abnormal outside the visual system There was a colour vision defect in his left eye and the visual acuity was 0 2 Ophthalmoscopy of the left eye showed very poorly defined papillomacular nerve fibres and a great number of narrow nerve fibre defects in the superior arcuate nerve fibre bundles (Fig 4) The visual field examination demonstrated a central scotoma and numerous slit scotomas corresponding to th small defects Ophthalmoscopy of his *asymptomatic* right eye showed a few narrow defects in both arcuate bundles There as a vaguely defined arcuate scotoma but no central scotoma Visual acuity as well as colour vision was normal The findings make *the diagnosis of multifocal demyelinating* neuropathy h ghly probable

Case 5 Male aged 24 Work up for a two year long history of intermittent headaches d sclosed bilateral papilledema and an absolute homonymous visual field defect to the left. Pneumoencephalography gave evidence of a huge mass lesion displacing the ven tricular sy tem to the left This mass proved to be a giant ophthalmic artery aneurysm The right internal carotid artery was ligated in the neck Recovery was uneventful There was partial restitution of the visual field defect but a homonymous grossly in congruent predominantly relative defect to the left remained The bilateral papilledema was replaced by partial optic atrophy The arcuate bundles of the retinal nerve fibre layer on the right were wasted as compared to the left eye The left optic disc was completely denuded from nerve fibres nasally (Fig 5 A B) The appearance of the nerve fibre pattern alone is indicative of axonal loss due to damage to the right optic tract (Hoyt & Kommerell 1973)

Comment

The cases reported here show an axonal loss due to various causes In some of these cases the visible nerve fibre layer defect was the first clue to ocular patho logy Therefore the examination of the nerve fibre layer is a valuable comple ment to other examination techniques The method is rapid and simple and requires care but no cooperation Sometimes red free light enhances the nerve fibre pattern In patients with myopia or poorly pigmented fundi it is difficult to evaluate the thickness and the structure of the nerve fibre layer These diffi culties are presently still more pronounced in cases with opacities in the optic media This prevents the successful application of this new clinical tool in many patients Also basic information on the anatomy of the human retinal nerve fibre layer is lacking Therefore it is not possible to explain the mechanisms underlying the appearance of focal and diffuse atrophy of the retinal nerve fibre layer

had disappeared and only a few exudates remained in the central part of the fundus.

Case 3 Female aged 62 On a routine eye examination *ophthalmoscopy* showed deep marginal inferior excavations in both eyes In the right eye a sharp border between a total focal nerve fibre atrophy and some remaining nerve fibres was found below the papillomacular bundle and a wedge shaped defect was seen in the superior arcuate bundle (Fig 3) Corresponding visual field defects were found on the tangent screen The intraocular pressure never exceeded 18 mmHg by applanation There was no history of haemodynamic crises The blood pressure was normal on repeated controls. No hypercoagulative state or vascular disease was found A diagnosis of bilateral low tension glaucoma was made

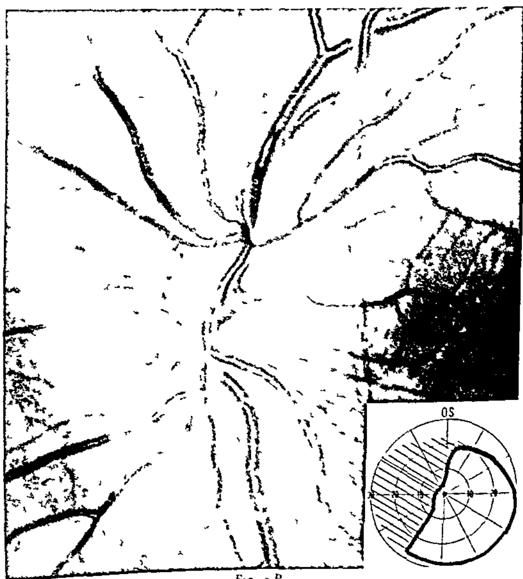


Fig 3B

Case 4 Male, aged 24 There was an 18 month s history of fluctuating visual loss in the left eye A neurological examination disclosed nothing abnormal outside the visual system There was a colour vision defect in his left eye and the visual acuity was 0 9 Ophthalmoscopy of the left eye showed very poorly defined papillomacular nerve fibres and a great number of narrow nerve fibre defects in the superior arcuate nerve fibre bundles (Fig 4) The visual field examination demonstrated a central scotoma and numerous slit scotomas corresponding to th small defects Ophthalmoscopy of his *asymptomatic* right eye showed a few narrow defects in both arcuate bundles There was a vaguely defined arcuate scotoma but no central scotoma Visual acuity as well as colour vision was normal The findings make the diagnosis of multifocal demyelinating neuropathy highly probable

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ENOPHTHALMOS CAUSED BY ORBITAL METASTATIC BREAST CARCINOMA

BY

R S MANOR

The case is described of a 72 year old woman with orbital metastatic breast carcinoma which presented as enophthalmos It is stressed that any change in the state of bulging of the eyes e g the appearance of a unilateral enophthalmos *may constitute a useful* indication of orbital metastasis in patients with breast carcinoma

Key words enophthalmos – orbital metastatic breast carcinoma – secondary glaucoma

From textbooks (Ingalls 1953) medical reports and everyday practice unilateral *exophthalmos* is known to suggest the possibility of a local space occupying lesion On the other hand the presence of enophthalmos is generally related to either 1) a post traumatic situation 2) a facial hemiatrophy or 3) a paretic lesion of the *sympathetic nerve (enophthalmos + relative myosis + slight* ptosis with narrowing of palpebral fissure the well known Claude Bernard Horner

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Fig 1
Photograph of the patient s eyes Note the right enophthalmos

Discussion

Just as the nipple of a breast affected by carcinoma may retract so the ocular globe may also become retracted when an orbital metastasis is present (Hughes 1979) It is possible that the collagenous material elaborated by the metastatic carcinoma contracts thereby producing the enophthalmos or that the orbital fat replaced by the tumor was of greater volume than the tumor itself (Walsh & Hoyt 1969)

In the case reported here the presence of defective orbital walls may constitute a supplementary explanation for the enophthalmos It is possible that

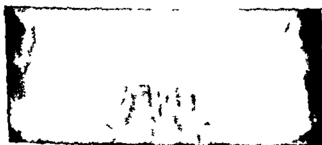


Fig 2
X ray of the orbitas *showing the destruction of the right parietal bone and of the right* lateral and inferior walls as well as the disappearance of the superior orbital fissure

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GRAFT THICKNESS AFTER PENETRATING KERATOPLASTY

BY

NIELS EHLERS

Graft thickness was studied in 70 patients subjected to penetrating keratoplasty. A postoperative swelling was followed by a decreasing thickness during 3–4 months, after which period the graft was found to be thinner than normal and in cases with a normal contralateral eye thinner than the cornea of that eye. During the following months the graft thickness increased and finally assumed the value of the contralateral cornea. Any complications during the postoperative course were immediately reflected in an increased thickness. Quantitative data supporting the above statements are given, treated statistically when adequate.

Key words: cornea – penetrating keratoplasty – graft thickness – pachometry – surgery

Under normal *in vivo* conditions a constant thickness of the cornea is maintained, but a number of circumstances, e. g. ocular inflammation, degenerations or surgery, give rise to increased thickness. A marked swelling may be observed in the slit lamp as folds in the posterior corneal surface, whereas smaller changes are revealed only by measurement of the thickness (pachometry). After keratoplasty this provides information of clinical as well as of theoretical interest.

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Material and Methods

Material The study comprised 40 patients subjected to 81 penetrating keratoplasties Thirty two of the cases were followed regularly through the entire postoperative course, while in the remainder a limited number of readings were taken

Surgical technique The cornea was removed from the donor and kept in a moist chamber at 4°C endothelial side up until used The average age of the donors was 36 years (range 14–64) and the total average post mortem age of the grafts was 5 5 hrs (range 0 50–19 25) All cases had a 7 0 mm punched out disc sutured by a 10-0 monofilament nylon and/or interrupted virgin silk sutures Air was injected to restore the anterior chamber Medication was given rigorously topical atropine chloramphenicol and steroid from the first postoperative day and from the 5th day systemic steroid starting with 80 mg prednisone daily reduced on the 10th day to 15 mg and then reduced gradually to zero over several months All operations and examinations were done by the author The pre operative diagnoses are summarized in Table IV The group other diagnoses includes traumatic lesions and various dystrophies

Table I
Central thickness of right and left cornea

	Right		Left	
	Reading along line of sight	Reading along line perpendicular to corneal surface	Reading along line of sight	Reading along line perpendicular to corneal surface
Mean (N = 15)	0 503	0 503	0 518	0 504
s e m	0 006	0 006	0 007	0 007
Measuring accuracy	0 0012	0 0010	0 0016	0 0011

The figures in the first line are the average values for 15 randomly selected members of the hospital staff The single values were determined as the mean of two readings

Standard error of mean is calculated from the conventional formula $\sqrt{\frac{\Sigma (x - \bar{x})^2}{n(n-1)}}$

The measuring accuracy is the standard error calculated from the series of double readings

The difference between the right and the left cornea is statistically significant for readings along the line of sight (paired comparison $P < 0.001$) but this side difference disappears when the readings are made along the line perpendicular to the corneal surface (paired comparison $P \sim 0.5$)

Pachometry Corneal thickness was measured with the Haag Streit pachometer modified according to Mishima & Hedbys (1968) The principle of the method has previously been discussed (Ehlers & Kruse Hansen, 1971) and normal reference values have been established (Kruse Hansen 1971 right eye 0.520 ± 0.002 left eye 0.524 ± 0.002 mm) When optical correction for perpendicular incidence is not employed a right left difference is encountered, which however disappears with correct optical geometry of measurement (Table I) The pin lights used to enable the slit beam to fall perpendicularly onto the anterior corneal surface (Mishima & Hedbys 1968) were only employed to determine the measuring position and were switched off during the alignment

Corrections for variation in horizontal corneal curvature have been made when appropriate, whereas no correction for variation in refractive index due to stromal swelling has been attempted The importance of changes in refractive index in the study of corneal thickness was emphasized by Arner & Rengstorff (1972) who calculated a 3 % increase in apparent thickness if the corneal refractive index was reduced to that of water Similarly from the refractive index of dry cornea reported by Fischer (1930) (1.384) a 1 % decrease in apparent thickness could be accounted for Thus theoretically the error due to index changes could be about 4 % but it seems safe to assume that in the cases met with in practice only much smaller changes occur (Fatt & Harris 1973) well below the measuring accuracy

Statistics Evaluation of the data was made by computer following the directions of Sokal & Rohlf (1969) Values are given as mean ± standard error of mean Significance evaluation was made with the *t* distribution when possible using the method of paired comparison

Results

In the immediate postoperative days a swelling of the graft was observed often with folds in the posterior corneal surface It was common to find slightly more oedema along the suture line The initial swelling was followed by a thinning which was at first rapid and then appreciably slower The following quantitative data all apply to central graft thickness

Postoperative swelling Maximum thickness was found within the first few post operative days The average value being 0.604 ± 0.016 mm (N = 36) The air injected to reform the anterior chamber probably explains that the maximum thickness was not always found on the very first postoperative day The maximum thickness was positively correlated to the age of donor (Table II) whereas a correlation between maximum thickness and post mortem age of the graft may only be suggested ($P < 0.1$) Fig 1 illustrates the correlation between maximum thickness and the age of the donor while Table III shows the maximum thickness in three groups according to post mortem age of the graft The age of the graft has little if any influence upon the postoperative swelling of the graft

Table II
Correlations for T_{max} and T_{min} of graft

Correlation	No of cases	Correlation coefficient r	t	p
T_{max}/Age of donor	36	0 488	3 11	0 01
T_{max}/Age of graft	36	0 303	1 78	0 1
T_{min}/Age of donor	29	0 047	0 24	n s
T_{min}/Age of graft	29	0 080	0 42	n s

$$t = r\sqrt{\frac{n-2}{1-r^2}}$$

With regard to preoperative pathology the 36 cases comprised 11 vascularized and 25 non vascularized corneas The average maximal thickness did not differ for these two groups (Table IV) The series could be grouped into nine cases of keratoconus five cases of herpetic keratitis eight cases of endothelial dystrophy and 14 cases of other pathology No significant differences could be demonstrated between the average maximum thickness in the various groups but it may be noted that the highest value was found in the group of endothelial dystrophy the lowest in the group of keratoconus (Table IV)

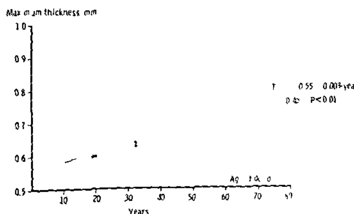


Fig 1
Maximum graft thickness plotted against age of donor

Table III

Influence of post mortem age of graft upon maximum graft thickness

Post mortem age of graft (hours)	Average of hours	No of cases	Average max thickness (mm)	s e m
< 4	1 71	20	0 642	0 019
4 12	7 40	12	0 692	0 024
> 12	17 25	4	0 694	0 079

The differences between the groups are not statistically significant

Course of thinning After a swelling within the first postoperative days the corneal thickness started falling and in successful cases leveled off in a few months An analysis of the studied cases suggests that two types of corneal thinning may be described In type 1 the thickness steadily falls at first rapidly then more and more slowly Characteristic examples are shown in Fig 2 In type 2 the fall in thickness is interrupted by a transient increase in thickness soon to be followed by a resumption of the steady fall Examples are shown in Fig 3 The increase in thickness can reasonably be interpreted as being due to an increase in endothelial permeability and would undoubtedly be considered

Table IV

Maximum thickness of graft and preoperative pathology

	No	Mean	s e m
vascularization	11	0 642	0 030
– vascularization	25	0 674	0 019
Keratoconus	9	0 630	0 035
Herpetic keratitis	5	0 640	0 045
Endothelial dystrophy	8	0 [illegible]70	0 032
Other diagnoses	14	0 663	0 023

The differences bet een the maximum thickness in the various groups are not significant For the largest difference (endothelial dystrophy – keratoconus) $t = 1\ 9$ $0\ 05 < P < 0\ 1$

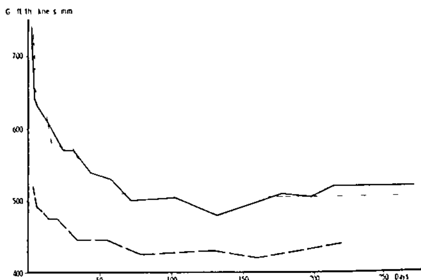


Fig 2
Graft thickness after penetrating keratoplasty steadily falling in the first postoperative months (type 1)

a complication were it not for its frequent occurrence A type 2 course was seen in 15 out of 32 regularly measured cases 15 cases were of type 1 one case was intermediary and in the remaining case the grafting was unsuccessful

The course of thinning could not be correlated to preoperative diagnosis vascularization degree of histocompatibility or to changes in steroid medication

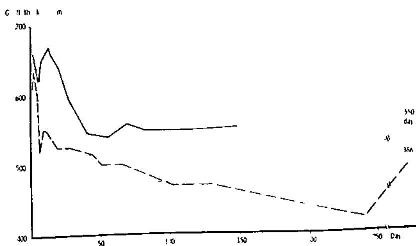


Fig 3
Graft thickness after penetrating keratoplasty showing a transient rise within the first weeks (type 2)

Late changes The corneal thinning leveled off at an average thickness of 0 486 ± 0 006 mm (N = 29) reached after an average of 111 days (s e m 10 9) the range being 14 to 240 days This minimum thickness is significantly lower than the normal average thickness of 0 520 ± 0 002 mm (Kruse Hansen 1971) ($P < 0.001$)

The minimum thickness was unrelated to age of donor age of graft (Table II) preoperative vascularization or to degree of histocompatibility

In 18 out of 32 cases followed regularly a subsequent increase in thickne s was seen as illustrated by Figs 2 and 3 while in eight cases no increase was seen within the observation period which was invariably longer than 6 months Finally in six cases no evaluation in this respect was possible due to graft rejection or recurrence of host disease (virus infection or endothelial dystrophy)

Final thickness In 31 cases with clear grafts after 1 year or more an average thickness of 0 573 ± 0 004 mm was found This value does not differ from the normal reference value

Seventeen cases with a normal contralateral cornea were observed for more than 6 months and thus allowed a comparison of this thickness of the patient with the final graft thickness The findings are illustrated in Fig 4 The diagram is a plot of the difference in thickness between the two eyes versus time after operation In the period 6–12 months after operation there is still some increase in thickness of the graft approaching that of the fellow eye For the right hand

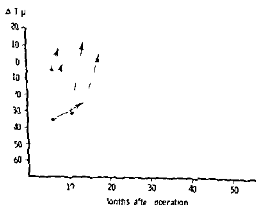


Fig 4

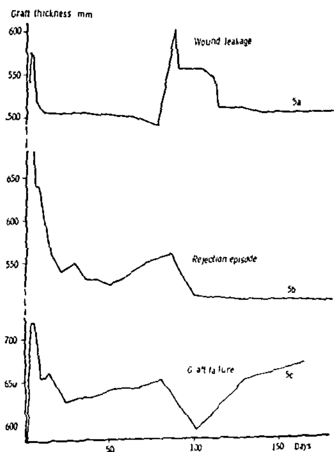
Thickness of graft n inus thickness of contralateral cornea (ΔT) plotted against time after perati n Each po nt represents one patient except for those connected by an arrow

Table V

Final thickness of grafts from 1 donor to 2 recipients and from 2 donors to 1 recipient

	No of cases	Equal thickness difference ≤ 0 005 mm	Unequal thickness difference > 0 005 mm
1 donor to 2 recipients	10	3	7
2 donors to 1 recipient	5	3	2

$\chi = 1\ 25\ 0\ 2 < P < 0\ 3$



Fig

Clinical conditions reflected by the graft thickness

group of 10 patients operated on more than 30 months earlier the average thickness of the grafts was 0 515 ± 0 008 and that of the contralateral corneas 0 516 ± 0 008 No statistically significant difference may be demonstrated even when tested by the method of paired comparison ($0 4 < P < 0 5$) The average thickness of the grafts and of the fellow eyes does not differ significantly from the normal average values

In 10 cases corneas from one donor were given to two recipients and in five cases both eyes of a patient were grafted Statistical correlation could neither be demonstrated between the thickness of graft 1 and 2 nor between the two grafts in bilaterally operated patients When grafts differing by 0 005 mm or less were considered to be of equal thickness the cases may be presented as in Table V It may be suggested that the grafts tend to assume the thickness of the host rather than to maintain the thickness of the donor However the difference is not statistically significant ($\chi^2 = 1 25$)

Complications and unsuccessful cases Complications during the immediate postoperative and later periods are sensitivity reflected in the thickness Among the mechanical complications wound leakage or rupture as well as iris adhesion to the wound and lens cornea contact are promptly followed by swelling of the graft (Fig 5a) Graft rejections are characterized by increased thickness An example is shown in Fig 5b The diagnosis of rejection was based on acute onset of oedema pain redness precipitates endothelial rejection lines and on a response to steroid medication Unsuccessful cases are characterized by constant or increasing thickness which in the observed cases preceded deep vascularization (Fig 5c)

Discussion

Corneal thickness after keratoplasty was first studied by Lavergne & Kelecom (1967) In 1969 Irvine Capella & Kaufman compared the thickness after keratoplasty with fresh and frozen tissue Brief reports on the subject were presented by Weekers & Pouliquen (1972) and by Zajacz (1972) The only larger study where the thickness was followed regularly after keratoplasty appears to be that of Ginsberg Capella & Kaufman (1972) who in 41 cases of penetrating keratoplasty followed the normal dehydration patterns in regard to donor materials and recipient disease state The corneal thickness was recorded during a 9 week postoperative course No statistically significant difference in results between disease states of recipient cornea or donor material were found

In the present study a regular finding was an initial phase of swelling The

maximum thickness was found within the first few days The maximum thick ness could be correlated positively to the age of the donor (Fig 1) and possibly *also to post mortem age of the graft* (Tables II and III) In this connection it should be noted that Forster & Fine (1971) found no statistically significant difference in results of penetrating keratoplasty due to donor age The maxi mum thickness was not correlated with preoperative *vascularization or host* disease although it may be noted without surprise that the largest postoperative swelling occurred in the cases with endothelial dystrophy

The deturgescense following the initial swelling has been described as being of two types one steadily falling and another with a transient rise in thickness A similar finding has not previously been reported The swelling is reasonably explained by an increase in endothelial permeability the nature of which how ever is obscure The transient rise often occurred in the second or third post operative week While later episodes of increased thickness may be regarded as rejection episodes it seems that those observed in type 2 thinning occur too soon after the operation to be due to a general sensitization and moreover they were never accompanied by other signs of rejection such as redness endothelial lines and increased aqueous flare There was no correlation between type of thinning and degree of histocompatibility in the studied series Endo thelial permeability undoubtedly is of importance for the thinning but factors such as biomechanical reorganization and stromal metabolism may also be considered

The average thickness after 3–4 months was 0 486 mm As it must be presumed that the preoperative donor cornea thickness was in the region of the normal average of 0 520 it must be concluded that after some months the graft has a thickness below normal In the following months the graft thickness increases and after about 1 year has reached a normal value very close to that of the recipient s opposite eye The explanation of this very slow decrease and increase *is unknown* but it could be thought of as being caused by alterations in the polysaccharide ground substance which are known to take place in wound heal ing (Anseth 1972)

It is in accordance with the concepts of corneal physiology that any complica tion (Fig 5) is followed by an increase in thickness mainly by affecting corneal endothelial function (Capella & Waltman 1971) and accordingly steroids are effective in restoring the normal thickness (Wind & Wood 1971)

From the present studies of corneal *thickness after keratoplasty and from* the reports in the literature it must be concluded that the graft thickness follows regular patterns which are valuable in the clinical evaluation of the single case Any complication is sensitively indicated by an increase in thickness which can be recorded objectively

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THE c-WAVE OF THE HUMAN D C REGISTERED ERG II CYCLIC VARIATIONS OF THE c WAVE AMPLITUDE

BY

KLAS OLAV SKOOG and SVEN ERIK G NILSSON

The *c* wave of the human ERG was studied with a new d c technique The *c* wave amplitude showed cyclic variations with a frequency of about 2/hour when repeatedly recorded The average amplitude difference between the peak and the trough of the first oscillation was about 150 µV With a stimulus frequency of 2/min the changes resembled damped oscillations After 1 hour they were quite small With a stimulus frequency of 0 5/min the oscillations were not clearly damped within 1 hour There was also evidence of a much slower change upon which the 2/hour oscillations seemed to be superimposed These findings will be of importance if a clinical routine procedure for registrations of the *c* wave is to be developed

Key words electroretinography – clinical method – *c* wave – retina – pigment epithelium

In a previous paper on the human d c registered ERG the *c* wave was studied quantitatively with a new method A linear relationship between the *c* wave amplitude and the logarithm of stimulus light intensity was found within the

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range studied (2 log units) (Skoog & Nilsson 1974) In the present paper the same method modified from Knave Nilsson & Lunt (1973) was used to investigate long term cyclic changes of the c wave amplitude on repeated stimulations

The literature on the c wave component of the human ERG is very limited compared to the large amount of publications about other components of the human ERG Kahn & Lowenstein (1924) Hartline (1925) Sachs (1929 1931) Cooper Creed & Granit (1933) obtained human ERGs with c waves apparently with great technical difficulties These authors did not state whether mydriatic drugs were used to eliminate iris potentials which may simulate c waves Wirth (1951) and Dodt (1951) also recorded the human c wave the latter with a d c technique When making several recordings during an experiment they noted that, as a result of light adaptation the c wave was present only in the first recording Hanitzsch Hommer & Bornschein (1966) and Heilig Thaler & Bornschein (1973) published a few d c recordings from human subjects during general anaesthesia The present technique for the first time gives stable and reproducible recordings of the human c wave without the aid of general anaesthesia The quality of the recordings which could be carried out repeatedly for very long periods of time made it possible to detect relatively small changes in c wave amplitude

When studying the sheep ERG Knave Persson Calissendorff & Nilsson (1973) and Calissendorff Knave & Persson (1974) observed that the amplitude of the c wave showed cyclic variations with time During preliminary work for a previous paper (Skoog & Nilsson 1974) it was found that also the human c wave amplitude changed in an oscillatory manner when repeatedly recorded This was important for the planning of the experiments concerning the intensity – amplitude relationship Since such time related changes of the c wave amplitude occurred regularly in all persons tested they will have to be considered if the present method is to be developed into a clinical routine procedure Consequently it was necessary to study these oscillations in more detail

Material and Methods

The recording procedure has been described in detail in previous papers (Knave Nilsson & Lunt 1973 Skoog & Nilsson 1974) This study was carried out on seven healthy volunteers females between 18 and 28 years of age Their pupils were dilated to 8 mm or more with 0.5 % tropicamide and 10 % metaoxedrine

given topically Thirty minutes of dark adaptation preceded the application of a scleral contact lens (for the tip of the recording electrode) to one of the eyes The tip of the reference electrode was connected to the forehead by means of a plastic chamber which was filled with Methocel®, as was the contact lens One of the arms was grounded The illumination of the eyes during application of the electrodes did not exceed 5 lux The free eye of the volunteer was made to fix upon a very weak deep red light in the ceiling during each registration A grounded wire net cage over the volunteer gave protection from external electrical disturbances Twenty min of further dark adaptation followed after the volunteer had been connected to the recording system

The signals from the eye passed saline bridges in agar filled polyethylene tubes and reached matched calomel half cells which served as recording and reference electrodes The impulses from the electrodes were fed into the differential inputs of a low drift d c amplifier They were lowpass filtered (220 Hz cut off 18 dB/octave) and reached a Hewlett Packard 5480 S signal analyzer The noise level of the electrode system was 5–10 μV and the d c drift 10–15 μV/h

The stimulus light with an approximately flat spectral emission curve which was produced by a 150 Watt ozone free Osram XBO xenon lamp passed heat reflection and heat absorbing filters (Zeiss) and neutral density filters (Balzer) with which the light intensity could be varied The intensity eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log relative intensity 0 A quartz fibre optics (Schott) was used to lead the light to the eye

The stimulus intensity used in this series of experiments was 4 5 relative log units Stimulus duration 1 sec Stimulus intervals 30 sec and 2 min respectively Intervals were not changed during an experiment Repeated registrations were carried out for at least 60 min Each recording lasted 5 sec or more thus always allowing the peak of the *c* wave to be identified Four recordings were averaged in experiments with the short stimulus interval and two recordings were averaged when the long stimulus interval was used In all 20 experiments were carried out

Results

With a stimulus duration of 1 sec and stimulus intervals of 30 sec the amplitude of the *c* wave of the human d c registered ERG showed cyclic variations as demonstrated in Fig 1 In the experiment illustrated the absolute values of the *c* wave amplitude measured from the base line varied between about 200 and 330 μV The variations diminished as the experiment progressed and resembled damped oscillations with a frequency of about 2 hour After 1 hour the oscilla

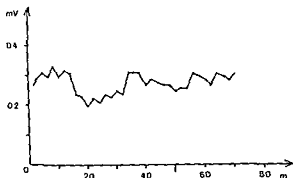


Fig 1
The cyclic chan es of the amplitude of the human c wave, measured from the base line on repetitive stimulations Stimulus interval 30 sec Light intensity 4 rel log units Flash duration 1 0 sec Four responses averaged

tions were generally quite small In Fig 1 the c wave amplitude levelled off at a value slightly below 300 μV In all experiments performed the frequency of the oscillations was always very close to 2/hour However in some experiments the oscillations were less damped than in Fig 1 The amplitude difference between the peak and the trough of the first oscillation was about 150 μV on n average

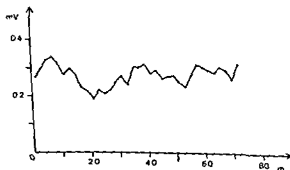


Fig 2
The cycl chang s of the ampl tu le of the human c wave measured from the b ttom of the pre ed ng trough on repetitive stimulations Recording cond tions the same as in Fig 1

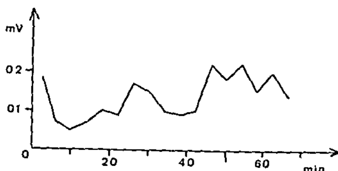


Fig 3
The cyclic changes of the amplitude of the human *c* wave measured from the base line on repetitive stimulations Stimulus interval 2 min Light intensity 4 5 rel log units Flash duration 1 0 sec Two responses averaged

Oscillations of the same kind were found also when the *c* wave amplitude was measured from the bottom of the preceding trough which is shown in Fig 2 (Figs 1 and 2 from the same experiment)

Changing the stimulus interval to 2 min did not affect the frequency of the oscillations (Fig 3) The amplitude of one oscillation also remained about the same However with this longer stimulus interval the oscillations were not significantly damped at least not within 1 hour This description was valid also when the *c* wave amplitude was measured from the bottom of the preceding trough

In many of the experiments it seemed that the oscillations described above were superimposed upon a much slower change but the duration of each experiment was not long enough to demonstrate the exact nature of this change

The amplitude of the *a* wave and the *b* wave did not change significantly when repeatedly registered in this investigation

Discussion

A light intensity of 4 5 relative log units above the *b* wave threshold and a stimulus duration of 1 sec were chosen since such stimuli were well tolerated As shown in preliminary tests repeated flashes of this kind did not diminish the *c* wave amplitude as a result of immediate light adaptation even at a stimulus interval of 15 sec

Variations of *b* wave amplitude in the individual case in recordings from different occasions were described by Karpe (1945) Peterson (1968) and others These variations were explained by the measuring and calibration errors and by differences in dark adaptation (Peterson 1968) Ronchi Del Signore & Brancato (1969) repeatedly recorded the *b* wave during the course of the day nd found a variation in amplitude in the same subject These authors suggested a possible existence of a rhythmical time change in *b* wave amplitude In experiments with the monkey Gouras & Carr (1964) measured the amplitude of relatively fast ERG responses obtained by means of a flickering light with a frequency of 4/sec When these amplitudes were plotted against time a slow oscillation with a frequency of approximately 2/hour was evident The phases of this oscillation correlated well with similar changes in the simultaneously recorded standing potential (SP)

In contrast to the reports cited above the present study describes time related changes of the slow *c* wave component To our knowledge such changes have not previously been reported for the human ERG A 2/hour oscillation of the amplitude of the human d c registered *c* wave was found With 30 sec stimulus intervals it was damped The same kind of 2/hour oscillation of the *c* wave amplitude was also shown in experiments on sheep (Knave Persson Calissendorff & Nilsson 1973 Calissendorff Knave & Persson 1974) where it seemed to be superimposed upon a slower cyclic variation with a frequency of 0 3 0 5/hour A very slow change could be traced also in the present registrations from human eyes but the duration of the experiments was not long enough to prove its cyclic character However the cause was not drift of the base line Calissendorff Knave & Persson (1974) also demonstrated that the *c* wave oscillations were dependent on the frequency of the stimulus flashes

The pigment epithelium is considered to be the source of the *c* wave (Noell 1954 Brown & Wiesel 1961 Steinberg Schmidt & Brown 1970) and the major origin of the SP (Noell 1954 Heck & Papst 1957 Gouras 1969) This may be an explanation for the similar time related changes which occur in the *c* wave amplitude and in the electrooculogram (EOG) The latter which reflects the SP shows oscillations with a frequency of about 2/hour (Kris 1958 Kolder 1959 Arden & Kelsey 1962a Gouras 1969 Taumer et al 1974 and others) By using EOG Jacobs Feldman Rabinowitz & Bender (1973) studied the human SP during sleep and found large changes during the first 30 min Between 1 5 and 6 hours the potential increased in a linear manner Very slow changes of the EOG potential were reported by Davis & Shackel (1960)

The [illegible] ave oscillations probably reflect changes in pigment epithelial and perhaps retinal metabolism On the basis of experiments with diseased eyes Arden & Kelsey (1962b) suggested that the oscillations of the EOG occurred in

the pigment epithelium Calissendorff Knave & Persson (1974) proposed that the oscillations of the *c* wave in sheep could perhaps depend on changes in rod sensitivity whereas they found it less likely that the changes were induced by the retinal and retinol molecules released from bleached rhodopsin The *c* wave is considered to be rod-dependent (Steinberg Schmidt & Brown 1970 and others) Changes in illumination prior to the registrations provoke EOG oscillations (Kolder 1959) and may therefore exert influence also on the *c* wave In the present study the major change in illumination occurred when dark adaptation was begun Considering also the time required for the application of electrodes etc more than 1 hour passed from the start of dark adaptation to the beginning of the registrations Most of the oscillations in the EOG provoked by the start of dark adaptation should be damped after 1 hour but some influence from these EOG changes cannot be fully excluded However if one wishes to develop a clinical routine procedure it is unrealistic to require more than 1 hour of dark adaptation Instead the technique should be standardized so that results from different persons or from the same person on different occasions can be compared the influence from previously started EOG changes or *c* wave oscillations being the same and as small as possible

The *c* wave oscillations require consideration if one wishes to obtain standardized measurements of the *c* wave amplitude in the clinic Preliminary tests on patients showed technically good results with a stimulus interval of 30 sec In clinical practice one could make averages of a large number of registrations along the oscillations or one could wait for the first oscillatory peak to appear It would probably be unrealistic to wait for a kind of steady state by stimulating repeatedly for more than 1 hour

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THE c WAVE OF THE HUMAN D.C. REGISTERED ERG
III. EFFECTS OF ETHYL ALCOHOL ON THE c WAVE

BY

KLAS-OLAV SKOOG

The amplitude of the *c* wave of the human d.c. registered ERG is known to oscillate with a frequency of about 2/hour in response to repeated stimulations. A small oral dose of ethyl alcohol caused a marked increase in amplitude of the oscillations and also elevated their mean level. A first peak was reached 10–15 min after the intake of alcohol. The *b* wave increased slightly in response to ethanol, but no significant effect on the *a* wave was observed with the present doses and stimulus conditions. The results encourage further studies of drug influence on the *c* wave with the intention of correlating ocular toxicity of a substance to *c* wave changes.

Key words: electroretinography – ethyl alcohol – *c* wave – clinical method – retina – pigment epithelium.

The main characteristics of the *c* wave component of the human d.c. registered ERG were recently described by Skoog & Nilsson (1974 a, b). The *c* wave was recorded quantitatively with a new method, modified from Knave, Nilsson &

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Lunt (1973) The *c*-wave amplitude showed cyclic variations with a frequency of about 2/hour when registered repeatedly The relationship between *c* wave amplitude and log stimulus light intensity was linear within the range studied (2 log units) Knowing these basic features it should be possible to evaluate the effects of various drugs and different pathological conditions on the human *c* wave The present method gives stable recordings not only from trained volunteers but also preliminary registrations from untrained patients are technically very promising General anaesthesia is not needed which is of particular value in studying drug effects on the *c*-wave since this precludes any interaction between the test substance and general anaesthetics Granit (1933) demonstrated that the *c*-wave of the cat is very sensitive to ether anaesthesia and Knave Persson & Nilsson (1974 b) obtained a clear decrease in the *c* wave of the sheep after a small intravenous dose (< 10 mg/kg) of an ultra-short-acting barbiturate thiopental (Pentothal-Sodium®)

The effect of ethyl alcohol on the *c* wave of the sheep LRG was investigated by Knave Persson & Nilsson (1974 a) A large increase of the *c* wave amplitude followed intravenously injected ethyl alcohol As to the human ERG the effects of ethyl alcohol on the *a* and *b* waves of the conventional ERG are known through the work of Ikeda (1963) Jacobson Hirose & Stokes (1969) and others but the author is not aware of any previous papers concerning the behaviour of the human *c* wave after ethanol administration In the present study the influence of ethyl alcohol on the human *c* wave was recorded without any disturbance from general anaesthetics Ethanol proved to be a suitable substance for demonstrating that the human *c* wave can be greatly influenced by drugs Large changes in *c* wave amplitude were provoked even by small oral doses The findings encourage further studies of the effects on the *c* wave of acute and chronic exposure to different compounds

Material and Methods

The recording procedure has been published in more detail elsewhere (Knave Nilsson & Lunt 1973 Skoog & Nilsson 1974 a b) In the present investigation the method was modified by the use of a digital tape recorder and in one experiment a suction contact lens

Five healthy volunteers one male and four females aged 24–28 years and weighing 56–70 kg were chosen 0 5 % tropicamide and 10 % metaoxedrine

given topically dilated the pupils to 8 mm or more The subjects were dark adapted for 30 min before the application of the electrodes The tip of the reference electrode was in contact with the forehead by means of a plastic chamber A scleral contact lens connected the tip of the recording electrode to one of the eyes The chamber as well as the contact lens were filled with Methocel® (Baeschlin) (In one of the experiments a modified contact lens equipped with a second polyethylene tube filled with saline and ending in a small beaker with saline was used The saline surface was placed 20 cm below the level of the eye. In this way a gentle suction was applied Applanation tonometry before the insertion and immediately after the removal of the lens showed no significant changes in intraocular pressure This contact lens will be further described elsewhere (Skoog 1974)) One of the arms was grounded The free eye of the volunteer was made to fix upon a very weak deep red light in the ceiling during each registration After the preparation procedure during which the illumination of the eyes did not exceed 5 Lux the volunteer was again kept in total darkness for 20 min before starting the registrations Saline bridges in agar filled polyethylene tubes passed the impulses from the eye to matched calomel half cells which served as reference and recording electrodes These were connected to the differential inputs of a low drift d c amplifier The signals were then fed into a Tandberg digital tape recorder and a Hewlett Packard 5480 S signal analyzer after lowpass filtering (220 Hz cut off 18 dB/octave) The noise level of the electrode system was 5–10 μV and the d c drift 10–15 μV/hour The volunteer and the electrodes were surrounded by a wire net cage which gave protection from external electrical disturbances

Stimulus light was produced by a 150 Watt ozone free Osram XBO xenon lamp It passed heat reflection and heat absorbing filters (Zeiss) and its intensity was varied with neutral density filters (Balzer) The intensity eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log relative intensity 0 The light passed to the eye through a quartz fibre optics (Schott)

A stimulus intensity of 4 5 relative log units was chosen One sec flashes were delivered with intervals of 30 sec Each recording lasted 5 sec or more thus always allowing the peak of the *c* wave to be identified Five registrations were averaged

The *c* wave amplitude was measured from the iso-electric line directly on the oscilloscope during the experiment. However *a* and *b* wave amplitudes were measured afterwards (from the iso-electric line) by displaying on the oscilloscope screen the information on the tape, using a faster sweep In this way also these comparatively fast components could be measured accurately Repeated registrations were carried out for 0–100 min Twelve experiments were performed

Ethyl alcohol was given in the form of brandy with an addition of 95 vol % ethanol to obtain 50 vol %. 0.4 g ethanol/kg body weight was given orally. The dose was taken in less than 3 min. Registrations were stopped temporarily during the ingestion of alcohol, but electrodes were kept in place and light stimulation continued twice a minute. The volunteers had been kept fasting for at least 3 hours prior to the experiment. Before that they had only a very light meal (e.g. a sandwich and a glass of juice). They were not under the influence of any previous intake of alcoholic beverages. The analyses of the blood alcohol concentration (ultramicro determination, ADH) were carried out at the Department of Alcohol Research, Karolinska Institutet, Stockholm.

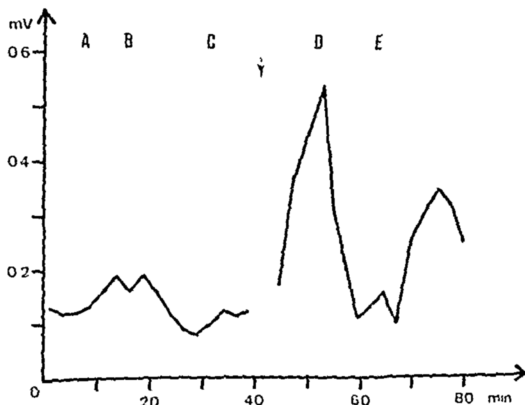


Fig. 1

A typical experiment where repeated registrations of the c wave amplitude (measured from the iso electric line) were performed before and after the oral administration (arrow) of 0.4 g ethanol/kg body weight. Stimulus interval 30 sec. Flash duration 1.0 sec. Light intensity 4.5 rel. log units above *b* wave threshold. The photographs of the ERGs taken at A, B, C, D and E are shown in Fig. 2.

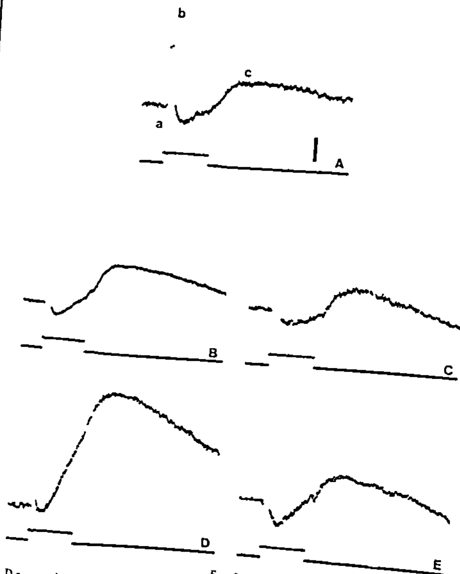


Fig 9

D c record ngs fron the experiment illustrated in Fig 1 Photographs taken at A B C D and E as ndicated in F g 1 A C, D and E are single recordings For comparison B is an a rag of five registrations Amplitude calibration 100 μV Stimulus duration 1 0 sec indicated on lower line

Results

Repetitive stimulations with 1 sec flashes twice a minute caused an oscillatory change of the *c*-wave amplitude as can be seen in the left part of Fig 1 A first peak occurred after about 15 min followed by a trough at about 28 min A second peak was building up or was just reaching its maximum when at 40 min (arrow) after the onset of recording a single dose of 0 4 *g* ethyl alcohol/kg body weight was administered orally As a result a marked increase in *c* wave amplitude with a maximum 12 min after the beginning of the alcohol intake was seen This peak was about three times as high as the first peak During the remaining part of the experiment the *c* wave amplitudes oscillated around a substantially higher mean level than before Moreover it seemed that ethanol caused a phase shift of the oscillations Figs 2 A B C D and E show d c registered ERGs including the *c* wave from different stages of the experiment illustrated in Fig 1 The very high *c* wave shortly after the intake of alcohol

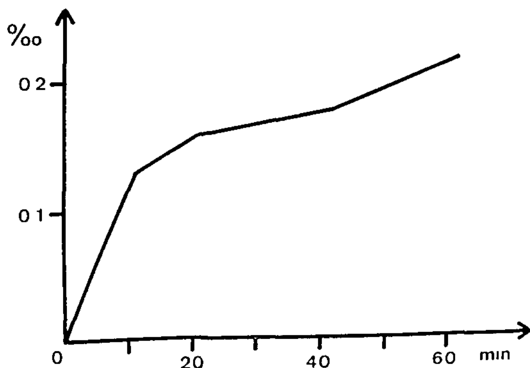


Fig 3

Blood alcohol concentrations in per mille (w/w) after the oral intake of 0 4 g ethanol kg body weight by a volunteer Alcohol was given at time 0

is seen in Fig 2 D Fig 3 demonstrates the blood alcohol concentrations from the same volunteer under identical fasting conditions and after the same dose of alcohol It may be seen that the blood alcohol concentration rose throughout the first hour after the intake of ethanol The fastest increase took place during the fir t 11 min There was a moderate increase in *b* wave amplitude after alcohol while no significant alteration in *a* wave amplitude was found The other experiments not shown here fully confirmed the above findings

Discussion

The effect of ethyl alcohol on the *a* *b* and *d* waves of the ERG has been studied in several animal experiments A decrease in *a* and *d* wave amplitudes was found by Bernhard & Skoglund (1941) (frog) Tomita Funaishi & Shino (1951) (frog) Forbes Burleigh & Neyland (1955) (frog) and by Kuriyama & Jojima (1957) (toad) An increase in *b* wave amplitude was described by Bernhard & Skoglund (1941) (frog) Forbes Burleigh & Neyland (1955) (frog) Manfredini & Trimarchi (1968) (rabbit) and by Morita (1970) (rabbit) Bernhard Knave & Persson (1973) reported that ethanol initially did not change the *a*-wave of the sheep ERG but a decrease was seen at higher alcohol concentrations The *b* wave amplitude increased to a maximum and then diminished in response to further increasing blood alcohol levels Applying a recently published component analysis of the low intensity ERG (Knave Møller & Persson 1972) the authors proposed that the isolated receptor response was not affected but that the positive d c response increased after the administration of ethyl alcohol

The *a* wave of the human ERG was not changed by orally given ethanol according to Ikeda (1963) but Jacobson Hirose & Stokes (1969) found an increase of the *a* wave amplitude after *i v* injection of ethyl alcohol A rise in *b* wave amplitude was reported after orally given (Straub 1957 Ikeda 1963) as well as after *i v* injected ethanol (Jacobson Hirose & Stokes 1969) The findings of the present study are in agreement with those of Ikeda (1963) However an effect in the *a* wave might have occurred with *different doses or different* routes f administration

The *c* wave has been found to be sensitive to a number of substances e g adrenaline (Therman 1937) sodium azide and sodium iodate (Noell 1954) and the antituberculous drug rifampicin (Knave Persson Calissendorff & Nilsson 1974) Recent experiments on sheep (Knave Persson & Nilsson 1974 a) showed a large increase in *c* wave amplitude after the intravenous injection of ethyl alcohol which also influenced the standing potential (SP) of the eye After

a short negative d c shift the SP increased and reached a maximum after about 8 min The time course of the SP changes was similar to that of the *c* wave effects

The present investigation demonstrates a marked increase of the human *c* wave amplitude in response to ethyl alcohol The maximum effect appeared 10–15 min after an orally given dose The dose of alcohol could not have been completely absorbed by then Alcohol given orally is first absorbed very fast and then more slowly Considerable redistribution takes place among body compartments (Haggard Greenberg & Lolli 1941 Goldberg 1943) The influence of a certain blood alcohol concentration on test parameters in psychophysical experiments (Goldberg 1943 and others) was greater when concentrations were rising than when they were diminishing These facts might partly explain the timing of the changes in *c* wave amplitude after the administration of ethanol

The human *c* wave amplitude is known to oscillate with a frequency of about 2/hour when recorded repeatedly (Skoog & Nilsson 1974 b) and in the present experiments such oscillations were observed before alcohol was given The administration of a small dose of alcohol seemed to provoke a series of oscillations with about the same frequency but with a considerably higher amplitude and at a higher mean level

The mechanism of the ethanol effects on the *c* wave reported here cannot be determined only on the basis of the present experiments The pigment epithelium is generally considered a major source of the *c* wave which seems to be receptor dependent (Noell 1954 Brown & Wiesel 1961 Steinberg Schmidt & Brown 1970 Schmidt & Steinberg 1971) Consequently an increase in *c* wave amplitude could occur as the result of a change in pigment epithelium activity or in the receptor pigment epithelium relationship as suggested by Knave Persson & Nilsson (1974 a) Ethanol influences the membrane potential of frog muscle fibres (Knutsson 1961 Knutsson & Katz 1967 Inoue & Frank 1967) lobster axons (Houck 1969) and *Aplysia* neurons (Bergmann Klee & Faber 1974) A membrane effect on pigment epithelium cells is not unlikely An increase in *c* wave amplitude might also be caused by a decrease of a slow negative component of the ERG Bernhard & Skoglund (1941) recording from frog eyes proposed that alcohol selectively suppressed P III of Granit s analysis (1933) thereby diminishing the *a* wave and increasing the *b* wave The work of Murakami & Kaneko (1966) Pautler Murakami & Nosaki (1968) Hanitzsch & Trifonow (1968) Murakami & Sasaki (1968 a b) Ernst & Arden (1972) Knave Møller & Persson (1972) and Hanitzsch (1973) indicate that P III consists of at least two subcomponents both in cold blooded vertebrates and in mammals a distal part from the receptors and a proximal part from the inner nuclear layer Bernhard Knave & Persson (1973) recorded the low intensity ERG of the sheep

and noted th[illegible] the receptor potential was unaffected by alcohol. Moreover Murakami & Sasaki (1968 a b) stated that ethanol suppressed the proximal P III of the carp Therefore it cannot be excluded that at least some fraction of the increase in *c* wave amplitude after alcohol administration is the result of a decrease of a negative slow potential from the neuroretina It should be interesting to study the effects of alcohol on the potentials from an eye treated with sodium iodate which is known to destroy the pigment epithelium (Noell 1954 and others) Such an experiment was performed with sodium iodate and barbiturate (Knave Persson & Nilsson 1974 b) also including a correlation of electroretinographic and ultrastructural findings (Nilsson Knave & Persson 1974)

Since the pigment epithelium builds up part of the standing potential (SP) of the eye (Noell 1954 Heck & Papst 1957 Gouras 1969) and is considered to be a major source of the *c* wave (see above) it should be interesting to compare drug effects on the *c* wave with those on the SP This was done in sheep eyes with alcohol and thiopental by Knave Persson & Nilsson (1974 a b) The present method for the quantitative d c registration of the human *c* wave makes it possible to investigate the influence of drugs on this ERG component A new procedure for the direct recording of the human SP with the aid of a suction contact lens has recently been developed (Skoog 1974) to serve in comparative studies as indicated above

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Mogens S Vorn External Eye Methods of Examination Scriptor DK 1656 Copenhagen 1974 200 pages, Price Danish kroner 210

The author needs no introduction to the readers of *Acta* He is well known to all of us through his about 100 articles published in *Acta Ophthalmologica* A comprehensive knowledge and great energy are required to be able to write such a brilliant book describing methods of examination of the external eye In this book we find collected the facts which students of ophthalmology have hitherto been obliged to search for in many different works viz an accurate description with detailed references examinations to be undertaken with a view to diagnosing and treating external eye diseases The book contains numerous illustrations colour photographs and Dr Norn s own instructive line drawings

It is a book of inestimable value to all ophthalmologists equally useful to those about to start their studies within this special line and as a reference book for ophthalmic medical practitioners

I can hardly find any item to criticize in this work which deserves a wide circulation

P M Møller

Symposium on Surgery of the Orbit and Adnexa Transactions of the New Orleans Academy of Ophthalmology C V Mosby Company Saint Louis Price US $ 34 15

Once a year since 1964 the New Orleans Academy have published the transactions of symposia on various important specific subjects This year the subject is orbital diseases and their treatment In recent years considerable advances have been made in this area of ophthalmology especially in terms of therapy

Contributors to this book include some of the best known names in ophthalmology in the United States (Beard Blodi Callahan Ellsworth Jones Keeney and Wadsworth)

On perusal one is aware of a superior knowledge about these subjects which verge on neighbouring disciplines Nothing seems to have been left out from extremely mutilating trauamas and malignant orbital tumours to more benign conditions such as diseases of the lachrymal canal malalignment of the eyelids ptosis and external congenital eye anomalies

Each chapter describes not only the pathology and pathogenesis but also the latest principles in treatment and here one has the impression that in the United States ophthalmologists treat many case which we in Denmark would traditionally send to specialists in other departments (plastic surgery jaw surgery and neurosurgery)

The book is well written with an excellent classification of subjects and the text is supplemented with an abundance of illustrations which include both photographs and skilful line drawings

It is not easy to recommend any one particular section more than any other but the results of the treatment of rhabdomyosarcoma seem most impressive

The book concludes with a report from a Round Table discussion this chapter makes for profitable reading as it answers many of the questions one would have liked to have put forward oneself

The table of contents is thorough and easy to use All in all the book can be warmly recommended for the library of any Eye Department

H H Seedorff

Advice and Dissent in Glaucoma

Plaza Square Hyatt Union Square Hotel San Francisco Wednesday December 4 through Friday December 6 1974 Using two panels of distinguished experts current concepts of the physiology pathology and pharmacology of the glaucomas will be discussed *Advice* will be *given on preferred* instruments for diagnosis and on preferred methods of treatment *Dissent* by panel members and audience will be welcomed This program is presented by the Department of Ophthalmology University of California School of Medicine San *Francisco* in cooperation with Extended Programs in Medical Education University of California School of Medicine San Francisco California It is acceptable for 21 hours towards California Medical Association and American Medical Association certification under Category 1 *Registration* Date Wednesday December 4 1974 Time 8 30–9 00 a m Place Plaza Square Ballroom Hyatt Union Square Hotel San Francisco California Fee $ 175 00 includes lunches and social hour Registration in advance by November 11 is advised to determine space needed $ 10 00 fee for refunds requested before course no refunds after course begins To apply for refund please return Registration Receipt *Program Co Chairmen* Robert N Shaffer M D John Hetherington Jr M D H Dunbar Hoskins Jr M D and Vincente Joeson M D Mail to Extended Programs in Medical Education Room 575–U University of California San Francisco California 94143

Fourth Congress of the International Society of Geographical Ophthalmology and the 2nd International Symposium for Metabolic Diseases of the Eye Monday 30th June Friday 4th July 1975 Edinburgh Scotland

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a) Development and congenital ocular anomalies and diseases b) Strabismus c) Delivery of ophthalmic services in different countries of the world with special reference to the use and training of paramedical personnel d) Free papers will be welcome All papers must be strictly on Geographical Ophthalmology and themes Papers can only be read by members of the Society or invited guests Abstracts of not more than 200 words must be received before January 31 1975 (triplicate with triple spacing and one inch margin on either side) They should be sent to The President Dr L L Cass P O Box 688 Fort *Smith* N W T Canada Non members may attend but may not participate in the programme Dues for membership $ 10 00 to be sent to account number 48–00117 at the Bank of Commerce Fort Smith N W T Canada *President* Dr Elizabeth Cass Box 688 Fort Smith N W T Canada *Vice President* Dr A I Kornzweig 1200 Fifth Avenue New York U S A *Executive Secretary* Dr Viggo Clemmesen Central Hospital 4700 Naestved Denmark

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President Dr Jules François Place de Smet de Naeyer 15 Gent Belgium *Secretary* Dr Heskel Haddad 1200 Fifth Avenue New York 10029 U S A *Treasurer* Dr Marvin Kwitko Suite 1 5591 Cote des Neiges Road Montreal Quebec Canada

Official Themes for Symposium
1 Carbohydrate metabolic defects particularly diabetic retinopathy 2 Hemoglobinopathies particularly sickle cell disease 3 Large molecule disorders particularly Refsum Syndrome 4 Genetic aspects of metabolic eye disease For all information re scientific programmes for this symposium apply to Dr Heskel Haddad 1200 Fifth Avenue New York 100 9 New York, U S A For all information re Congress s fees etc apply to *Convener and Treasurer* Dr R S Bartholomew Dept of Ophthalmology University of Edinburgh The Eye Pavilion, Chalmers Street Edinburgh Scotland EH3 9HA

ISCERG Symposium

Next symposium of the International Society for Clinical Electroretinography (ISCERG) will be held from the 21st to the 25th of April 1975 at Nof Ginossar at the Sea of Galilee Israel Main *topic* Functional and Deprivation Amblyopia another *topic* Electrodiagnosis of Retinal and Striate Function in Opacities of the Ocular Media In addition free *papers* *Organizer* Prof Edgar Auerbach M D *Director* Vision Research Laboratory Hadassah Medical Organization Jerusalem Israel

Ophthalmological Radiology
Radiology of the Eye the Orbit and Vision

Columbia University College of Physicians and Surgeons will sponsor a course on the radiology of the eye the orbit and vision on Friday and Saturday March 21 and 22 19 Dr Guy D Potter 632 West 168th Street New York New York 10032 is Director of the postgraduate course The course will deal with the radiographic diagnosis of conditions affecting the eye and vision, *including* the orbit, the paranasal sinuses and the sella turcica The role of routine radiography and special procedures including tomography ultrasonography angiography xenography and dacryocystography will be presented Tuition s $ 125 This course is accredited by the AMA Category I award for 14 hours

The Second International Symposium of Eye Surgery

will be held in Bologna from May 25th to 29th 1975 The subjects to be treated are ocular orbitary prosthesis implants strabismus and nystagmus detachment of the retina surgery of the vitreous What proved to be an extremely stimulating and instructive procedure during the First International Symposium will be followed now as well Mornings will be devoted to the live performance of new and varied surgical techniques executed by the most renowned surgeons on patients with the above mentioned cases afternoons will be given over to the presentation of related papers by outstanding ophthalmologists to be followed by discussions The symposium is of special interest to surgeons wishing to enhance their knowledge by witnessing new and advanced techniques used by surgeons from the international community Simultaneous translations will be available in English French Italian and German Registration Fee 150 dollars For particulars please contact Prof G Cristini II Simposio Internazionale di Chirurgia Oculare Clinica Oculistica Università Via Massarenti 9 Bologna Italy